Using Lacunarity to Characterize Pore Distribution in Scaffolds

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Abstracts

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Artificial Kidney—Uremic Toxins—Symposium

K1 (EI0154)

Ages in Hemodialysis: Tissue- and Plasma- Autofluorescence

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Objectives: Advanced glycation endproducts (AGEs) accumulate in all human subjects, e.g. in the skin. Because part of the AGEs shows autofluorescence, skin autofluorescence (SAF) generally increases with calendar age. SAF values above normal have been reported in patients with cardiovascular (CV) risk. SAF was a strong marker of CV mortality in hemodialysis (HD) patients [Meerwaldt, JASN 2005]. High levels of plasma AGEs were reported as well in HD patients compared to control subjects [Floridi, NDT 2002]. A consortium was formed to study the use of SAF for measuring the effectiveness of interventions that aim to decrease (CV) risk in dialysis patients. **Methods:** SAF was measured non-invasively with the AGE Reader (DiagnOptics Technologies B.V., Groningen, The Netherlands) at the inner forearm in HD patients: 33 (Umeå), 170 (Skopje), and 109 (Groningen). In Umeå and Skopje measurements were repeated at least twice a year for 15 resp. 24 months. In Umeå and Groningen measurements were performed before and after dialysis; plasma autofluorescence was determined in these patients before and after dialysis as well. **Results:** Plasma fluorescence decreased 12% ($p < 0.001$) during a dialysis session, whereas SAF did not change significantly. This confirms that SAF mainly represents tissue fluorescence. In all centers mean SAF was increased 40–60% as compared to healthy subjects. The yearly increase of SAF in Skopje was higher than in Umeå, and about ten times higher than in healthy subjects. Some variation in SAF during the year was observed in the Umeå data, which needs further investigation. **Conclusions:** High concentrations of plasma AGEs seem related to their increased accumulation in tissue, as is visible in the increased skin autofluorescence and rate of increase. By not being influenced by single dialysis sessions, SAF shows to be a useful marker for assessing AGE accumulation and studying the related CV risk in HD patients.

O1 (EI0222)

The Use of a Skin Age Reader to Evaluate Risk of CVD and Mortality in Dialysis Patients

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Objectives: To measure annual increase in skin autofluorescence (AF), a marker of accumulation of Advanced Glycation end products (AGEs) in the skin of hemodialysis (HD) patients and various plasma markers including heart-type fatty acid binding protein (H-FABP) in order to find factors that can predict the mortality of HD patients. **Materials and Methods:** One hundred sixty-nine HD patients were enrolled in a clinical prospective study. Skin AF was measured at 4 time points at approximately 6 months intervals. At the same time points the routine blood chemistry and plasma markers of oxidative stress (Superoxide Dismutase and Myeloperoxydase), inflammation (C-Reactive Protein: CRP), endothelial activation (intercellular Adhesion Molecule-1: ICAM-1 and von Willebrand Factor) and myocardial and kidney damage (H-FABP) were measured. The entire study lasted 32 months. **Results:** Skin AF was increased in HD patients, especially in those with diabetes, in which it showed to be the sole independent marker of the presence of cardiovascular diseases (CVD). The mean annual increase of Skin AF (ΔAF) was 0.15 ± 0.09 AU (mean ± standard error). Seasonal fluctuations in Skin AF with a mean of 0.31 ± 0.10 AU (mean ± standard error) were only present in patients with Hepatitis C. In the multivariate Cox regression analysis we found that age diabetes, hypertension, annual ΔAF and values of CRP, ICAM-1 and H-FABP at the start of the study were independent predictors of overall mortality. Strong predictors of CVD mortality were age, diabetes, male gender, annual ΔAF and H-FABP and albumins. Moreover, combined use of annual ΔAF and single measurement H-FABP gives even better results in the prediction of the CVD mortality risk than separate use. **Conclusions:** Annual ΔAF and single measurement of H-FABP are strong independent predictors of overall and CVD mortality in HD patients.

O2 (EI0191)

Does the Advanced Glycation End Products (Ages) Food Intake Influence Mortality in Dialysis Patients?

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Objectives: The diet is a source of AGEs. Nutrition recommendations on calories and protein intake for patients on maintenance chronic hemodialysis are already published. Data about the influence of the ingested amounts of AGEs on the mortality is still unavailable. Aim of study: to assess the impact of calories, protein and AGEs intake on mortality in dialysis patients. **Methods:** One hundred fifty patients of mean age 55.69 ± 13.5 years and dialysis vintage 8.9 ± 6.6 years were included in a prospective study. Patients were followed for 36 months, up until death, kidney transplantation, or until the end of the observational period. Dietary records for 7 days were obtained and calories and protein intake were calculated. Daily AGEs intake was estimated by Teresia Goldbaerg et al. (J Am Diet Assoc. 2004). AGE intakes of survived and deceased patients were compared with independent T- test. **Results:** In the time period of 36 months of follow-up 36 (23%) HD patients died and one had a kidney transplant. AGE food intakes didn't differ between the two groups of survived and died patients (9.3 ± 4.2 vs. 9.2 ± 3.6 MU/day, p = 0.868). We found borderline significance for the difference in calories intakes, in favor of the survived patients (31.2 ± 8.0 vs. 27.8 ± 5.8 Kcal/kg/day, p = 0.061). The two groups also didn't differ in the protein intake (1.31 ± 0.73 vs. 1.10 ± 0.50 g/kg/day, p = 0.240). **Conclusions:** Mortality of dialysis patients is probably affected by more powerful factors than AGE food intake. Further studies are needed to confirm the impact of all nutritional factors on the survival.

O3 (EI0192)

Skin AF and Food, Is There Any Relation?

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Objectives: To investigate the influence of nutrition especially Advanced Glycation End products (AGEs) intake on accumulation of AGEs in Hemodialysis (HD) patients. **Methods:** One hundred fifty-six HD patients were enrolled in this study. Skin Autofluoresence (AF) was used to measure the AGEs accumulation. The enrolled HD patients were asked to record their daily food intake over a period of one week. From these recordings daily calorie, protein and AGEs intake were calculated. Body mass index (BMI), as a measure of nutritional state, was calculated. The routine blood chemistry and plasma markers of oxidative stress (Superoxide Dismutase and Myeloperoxydase-MPO), inflammation (C-Reactive Protein-CRP), endothelial activation (intercellular adhesion molecule-1 and von Willebrand Factor) and myocardial and kidney damage were measured. **Results:** The mean protein, calorie and AGEs intake correlated with each other ($R = 0.56$ $p < 0.01$; $R = 0.36$ $p < 0.01$; $R = 0.33$ $p < 0.01$). We found that the AGEs and protein intake is highest in the third quintile (BMI 21.16–23.79 kg/m^2) whereas the calorie intake was highest at the fourth quintile (BMI 23.79–27.00 kg/m^2). In the multivariate analysis we assessed the contributors of the annual increase of skin AF. The independent contributors of the increase of the skin AF were: lower or higher BMI than the 4th quintile of BMI (23.79–27.00 kg/m^2), lower AGE intake as well as shorter HD vintage and higher annual increase of MPO. We analyzed the influence of BMI on the annual increase of skin AF and we found that the relationship between BMI and the annual change skin AF can be represented as a U-shaped curve. The lowest point of the U-shape curve is 31 kg/m^2. **Conclusions:** We found that being slightly overweight and having higher AGEs intake results in lower AGEs tissue accumulation. The correlation between the change of Skin AF and BMI is a U-shaped curve with a bottom at 31 kg/m^2.

O4 (EI0409)

How Can We Optimize Haemodialysis to Prevent from Ages

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Objectives: Glucose degradation products are produced progressively during the lifespan. As mentioned above this results in an accumulation over time. Such accumulation is best known from patients that suffer from diabetes mellitus. The aim was to investigate if skin AF and plasma-AF was influenced by haemodialysis (HD). **Methods:** Design: clinical prospective studies of haemodialysis patients. The first study was performed to investigate if haemodialysis may influence AGE accumulation in dialysis patients in general. Since skin-AF is a good representative for GDP accumulation in these patients. The patients included were on chronic HD. Glucose 5 mmol/l was used as part of the dialysis fluid. Various dialysers were used. More than 30 patients were included. Paired statistical analyses were performed. The power of the studies is estimated to be more than 80%. Skin AF was measured before and after HD. Plasma fluorescence was also measured before start and after HD. **Results:** The results showed that HD resulted in a significant reduction of plasma AF. This resulted in a second study to evaluate if there was a difference in the efficacy using either high flux dialyzers or low flux dialysers. The patients were randomized in a cross over design using either HF or LF dialyzer. Skin and plasma AF was done as in the study above. The results showed no significant difference in skin AF, using LF versus HF. **Conclusions:** This data shows that HD patients had a significant elevated skin AF. The GDPs, estimated by plasma and skin AF will be reduced by HD. The molecules removed seem to be of a lower molecular size. Therefore sufficient dialysis is important to reduce accumulation of GDPs. The effect seems enough by using LF dialyzers in this regard. The effect of diet and glucose-free dialysates has to be further explored.

Smart and Responsive Biomaterials—Symposium

K2 (EI0042)

Smart Biomaterials

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Smart polymers are able to respond to changes in their environment. Shape-memory polymers are an example for stimuli-responsive materials, which can change their shape on demand. Such Polymers are of interest for a variety of application areas including biotechnology and medicine. Only a few polymeric biomaterials are established in clinical applications to date and most of these biomaterials have not primarily been developed for biomedical applications. Many implants have initially been developed to fulfill a structural / mechanical function. Examples for this category of medical devices are surgical sutures, hip prostheses or hernia meshes. The predictability of the long term behavior of biomaterials in physiological environments became apparent as a major challenge. In this context experimental as well as computational tools are being developed to evaluate the mechanisms of polymer degradation. With increasing clinical experience it became furthermore apparent that one single function is not sufficient, but multifunctionality is required. Vascular stents, which initially were purely metallic devices with a specific structural function, have been further developed by adding polymeric coatings to improve their hemocompatibility, which were partially loaded with drugs to avoid restenosis. Presently, degradable stents are under development. In this presentation, the scientific challenges of combining several functions in one material are described and examples for dual and triple functional polymers are given. Finally, the application potential of theses biomaterials in regenerative medicine is outlined. Potential applications include smart implants or drug release systems inducing endogenous regeneration and scaffolds for tissue engineering applications.

O5 (EI0354)

A Compound Nerve Guide Construct for Peripheral Nerve Regeneration

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Objectives: Currently, autologous nerve grafts are the gold standard for repairing critical-size gap defects in peripheral nerves following accidents, injuries etc. However, this results in donor site morbidity and functional recovery is often unsatisfactory. Instead, one can use artificial conduits to guide nerve regeneration. The goal of this study is to develop such a construct to provide structural and biochemical cues promoting axonal growth. **Methods:** The nerve guide consists of an inner chitosan core having axially directed, continuous pores made using directional solidification and an outer shell of electrospun poly(caprolactone) (PCL) fibres. Chitosan solution in CH_3COOH was filled into copper molds and cooled down with defined cooling rate and temperature gradient. This resulted in unidirectional growth of ice crystals along the temperature gradient, which were removed later using freeze-drying forming a porous structure. This was then mounted on rotating collector and a layer of PCL fibres was electrospun to give the final construct. **Results:** The construct had a total diameter of 1.3 mm. The chitosan core had a diameter of 0.7 mm with a pore size of 40 µm. Thickness of the electrospun wall was 0.3 mm with a fibre diameter of 3–4 µm. Pore size of the chitosan core, as well as wall thickness and fibre diameter of the electrospun shell, can be tailored as required by changing the process parameters. **Conclusions:** A compound construct was produced to act as a nerve guide conduit for repairing nerve defects. In future studies we will characterise its regeneration potential *in vitro* and *in vivo*. These studies will elucidate if the construct provides structural and biochemical cues

to enhance peripheral nerve regeneration. This work is supported by funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for the Cluster of Excellence REBIRTH (From Regenerative Biology to Reconstructive Therapy) and the International Foundation for Neurobionic (to KHT).

O6 (EI0404)

Characterising Advanced Nanoporous Activated Mast Carbons for the Treatment of Kidney Disease

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Objectives: A range of medical grade, phenolic resin based, activated carbon adsorbents (ACs) have been developed which offer great potential for the removal of albumin bound and larger molecular weight biological toxins associated with the progression of kidney disease. MAST carbons combine the superior adsorptive capacity of ACs with uniquely tailored nanoporosity to augment the removal of uremic toxins which are poorly removed by current haemoperfusion systems. A proof of concept study was carried out in order to assess the impact of MAST AC form and porosity on biocompatibility and functionality with respect to the removal of key biological toxins associated with kidney disease. **Methods:** The physical properties of a range of MAST carbons in bead and monolithic form were assessed by scanning electron microscopy and porosimetry. Cell based assays were used to assess the cytotoxicity of carbon leachate. The removal capacity of the carbons for a range of uremic toxins associated with renal failure was assessed using spectrophotometric, HPLC and ELISA based analysis. **Results:** MAST carbons have a high surface area for adsorption and were distinguished from other commercial carbons by the presence of larger nanoporous domains. MAST carbons were not cytotoxic and were capable of removing significant amounts of the larger biological toxins not removed by purely microporous carbons alone. **Conclusions:** The surface structure and form of MAST ACs may be manipulated to produce a material which is highly suited to the removal of protein bound and high molecular weight biological toxins. In this way nanoporous MAST ACs may offer a therapeutic strategy to augment current haemodialysis based systems by reducing uremic toxicity effects associated with disease progression.

O7 (EI0276)

In Situ Capture of Endothelial Progenitor Cells (Epcs) on Vegf-Bound Devices: Surface Architecture and Cellular Responses

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Objectives: This study focuses 1) to develop surface architecture enabling in situ capturing of EPCs flowing in arterial bloodstream aiming at long-term nonthrombogenic potential of implanted devices (stent and small diameter artificial graft), 2) to determine *ex vivo* cellular responses including selective harvesting, cell adhesion and proliferation and shear-stress resistance potentials and activation of intracellular signal transduction pathways, and 3) to report preliminary *in vivo* cellular responses in porcine models. **Methods:** 1) Covalent bonding of molecules [vascular endothelial growth factor(VEGF) and two VEGF receptor antibodies and Tie-1 and -2 antibodies] on thin-layered vinyl alcohol-copolymer. 2) Culture of human mononuclear cells on these protein-bound substrates and histocytochemical analyses, 3) determination of hydrodynamic shear stress dependence of adhered EPCs and endothelial cells (ECs) by radial flow chamber technique, 4) implantation of stent and electrospun artificial graft in porcine models. **Results:** Proteins were covalently bound to the polymer surface via activation of hydroxyl group. Among molecules examined, only VEGF exhibited high adhesion and proliferation characterisitics similar to those of fibronectin, and a quite high differentiation potential (expression of surface markers specific for EC) with culture time. In addition, day-order continuous activation of intracellular transduction pathways (phosphorylation of VEGF receptor, FAK, ERK and Akt) was observed for ECs adhered on VEGF-bound substrate. Once adhered, high detachment resistance to laminar flow was observed under arterial shear stresses. Based on these results, preliminary implantation study in porcine models using ultrasonic-atomized stents and custom-design electrospun artificial grafts, both of which are surface-arcitectured with bound VEGF, was conducted. The results showed that cells adhered on blood-contacting surface expressed VEGF receptor. **Conclusions:** The target molecule defined in this study is VEGF. Surface-bound VEGF expresses high adhesion, proliferation, hydrodynamic shear stress resistantce and diffrentiation potentials. Simple but reliable surface fabrication technology may provide nonthrombogenic potential to implantable cardiovascular devices.

O8 (EI0111)

Resilient Amorphous Networks with Shape Memory Properties for Use in Medical Applications and Tissue Engineering

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Objectives: In tissue engineering, flexible, form-stable and resorbable elastomeric networks can be used to prepare scaffolding structures with most advantageous properties. Here we describe the synthesis and characterization of a series of amorphous photo-crosslinked networks with tunable thermal- and mechanical properties based on trimethylene carbonate and D,L-lactide. **Methods:** The (co)oligomers were synthesized by ring opening polymerization of the corresponding monomers in the presence of varying amounts of hexanediol, and then functionalized by methacrylation. Networks were obtained by UV crosslinking in the presence of a photoinitiator. Of the obtained crosslinked structures, the network properties and the thermal- and mechanical properties were assessed. The shape recovery behavior of the different networks was evaluated quantitatively. **Results:** Amorphous networks were prepared from macromers with different molecular weights in which the monomer molar ratios were varied between 0:1 and 1:0. This allowed tuning of the glass transition temperature and mechanical properties. The values of the toughness, ultimate tensile strength and elongation at break of flexible networks with glass transition temperatures above room temperature increased with increasing macromer molecular weights. Networks prepared from macromers with a TMC content of 0.4 to 0.6 had Tg values close to or below body temperature. These networks are especially interesting as they are relatively rigid at room temperature, and flexible with mechanical properties in the range of soft tissues at body temperature. From these materials, porous and non-porous devices were fabricated. At room temperature these devices are relatively rigid and can be implanted non-invasively in their temporary shape, while at body temperature they return to their original permanent shape to perform a desired function. **Conclusions:** These properties, and their biodegradability and elasticity, make these networks materials very well-suited for the preparation of self-deploying implants in medical applications like tissue engineering, drug delivery, stenting and the support of soft tissues.

Cardiovascular 1: Devices—General Session

O9 (EI0172)

One Year Cost Comparison between Cardiac Transplantation and LVAD Therapy.

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Objectives: Left ventricular assist devices (LVADs) are increasingly used in end stage heart failure, not only as bridging therapy but also more as destination therapy. The perceived cost of LVAD therapy is considered to be a limit in its application. **Methods:** Actual hospital invoices of 20 consecutive surviving transplant patients (HTX) and 21 consecutive surviving patients receiving an LVAD were reviewed. All in hospital costs starting from the date of operation until one year post discharge were collected. Hospital costs were defined as the sum of all reimbursed costs and the patient's own share. The cost of the donor heart was calculated as an average of the total costs of explantation and transportation fees. The ambulatory cost is a sum of the actual hospital invoices and the mean ambulatory drug cost per month. **Results:** The duration of the first hospitalization was significantly longer in patients receiving an LVAD (LVAD-Group: 44,9 ± 24,6 days versus HTX-Group: 24,5 ± 7,3 days; $p < 0,01$). The initial hospitalization cost was higher in the LVAD-group (LVAD-Group: €40793 ± 19660 vs. HTX-Group: €27439 ± 13889; $< 0,05$). The cost of the device was €69239 versus €7810 for the donor heart. Monthly ambulatory costs were higher in the HTX-Group (HTX-Group: €3036 ± 815 vs. LVAD-Group: €699 ± 598; $p < 0,001$). The overall cost after one year was higher in the LVAD-Group (LVAD-Group: €118420 ± 18791 vs. HTX-Group: €72935 ± 15253; $p < 0,001$) **Conclusions:** LVAD therapy is initially more expensive than heart transplantation, predominantly by the device cost. However, ambulatory cost of a transplanted patient is significantly higher per month, due to the need for immunosuppressive medication and its monitoring. Given that these higher monthly costs are fixed, in the long term LVAD therapy will become less expensive compared to transplantation.

010 (EI0428)

Implantable Biventricular Assist Device: First In Vivo Results

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Objectives: Right heart failure is a common complication in patients with LVAD therapy. To assist both ventricles with one device, a completely implantable, pulsatile BVAD with specific assistance for the left and right heart is to

be developed. **Methods:** The compact device includes two pump chambers that are alternately compressed by hydraulic fluid, using a high efficient electro-hydraulic energy converter. Hydraulic bearings enable enhanced lifetime of the gear. The flat design (Vol. 435 cc) allows for completely implantation. Differentiated ejection and acute control of the stroke volume is enabled by a control algorithm, monitoring the filling of the pump chambers and the heart frequency. New seamless pump chambers are optimized by CFX and flow measurements using non-Newtonian fluid. The surfaces of the pump chambers are textured to allow cell adhesion. The TET is verified in vitro, enabling complete implantation. **Results:** The performance and durability of the BVAD was tested in mock loops up to 145 days. With a frequency of 120 bpm, the BVAD generates 5.1 l/min output for each ventricle. A speed of 6000 rpm of the drive unit enables the maximum pump frequency of 180 bpm with a maximum flow of 7 l/min. The energy consumption of the pump is between 6 and 9 Watt. A nearly physiological flow field is generated in the new chambers. During filling phase, two recirculation zones similar to those found in the human left ventricle are observed. Increased shear rates up to 1500 1/s are observed downstream the inflow. No superior warming of the drive unit was measured. **Conclusions:** The developed BVAD combines the advantages of displacement pumps and rotary blood pumps to support patients up to a BMA of 1.8 m^2. Separate control of the stroke volume and triggering the BVAD's frequency to the heart should enable sufficient unload of the ventricles.

O11 (EI0131)

Evaluation of the Maglev Motor and Design the Centrifugal Blood Pump for Pediatric Artificial Heart

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Objectives: We have developed a Maglev artificial heart for use in infant patients, which is required small size compare to adult devices. The active magnetic bearing offers better biocompatibility and longer device lifetime of the artificial heart by eliminating any actual physical contact. In this paper, a miniaturized self-bearing motor and centrifugal blood pump for pediatric artificial heart are reported. **Methods:** The self-bearing motor consists of a top stator, a bottom stator and a levitated impeller set between the both stators which have identical structure. The impeller is suspended axially with a double stator mechanism to enhance a motor torque with smaller device size. The motor regulates an axial position and a rotating speed of the impeller by using vector control algorithm. A target pump performance of the pediatric artificial heart is set as a flow rate of 1 L/min against a head pressure of 100 mm Hg. The pump has been designed with the computational fluid dynamics simulation. **Results:** A diameter and a height of the developed motor are 24 mm and 43 mm, respectively. The volume of the artificial heart is 21 mL. The motor can produce an attractive force of 16 N with an air-gap of 1.5 mm, and a rotating torque of 13 mNm with the impeller speed of 4000 rpm. From the results of simulation, an impeller speed of 4000 rpm is required to achieve the target head pressure and the flow rate. At this time, an axial thrust force of 0.14 N and a torque of 3.6 mNm act the levitated impeller. **Conclusions:** The large attractive force and the rotating torque indicate enhanced magnetic suspension and rotation performance for a smaller size and larger air-gap. This developed maglev motor is suitable for use in the pediatric artificial heart.

O12 (EI0122)

Comparison of 50 cc Penn State VAD Designs Using Particle Image Velocimetry

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Objectives: Congestive heart failure remains a major cause of death worldwide. We continue to develop and study ventricular assist devices (VADs) in an effort to assist these patients. As these devices miniaturize, thrombosis remains a major concern. Our current focus is to compare two pulsatile VAD designs using *in vitro* optical measurments to focus on the local wall shear rates in areas that may be prone to thrombus deposition. Furthermore, we compare the effect of heart rate variabililty on the local flow and its potential impact on said deposition. **Materials and Methods:** To measure and calculate the local wall shear rates in two acrylic model 50 cc VAD designs, particle image velocimetry (PIV) was used for heart rates from 75–150 beats per minute and the appropriate systolic duration. A standard mock circulatory loop and blood analog were used to simulate the cardiovascular system. Magnification of 12 microns per pixel was achieved for the PIV system and an error analysis performed. Multiple planes of PIV data were collected and wall shear rate maps produced. **Results:** The flow patterns for each device were not significantly altered when the heart rate increased. The major effect was an increase in velocity and subsequently, wall shear rate. Roughly speaking, the magnitudes of the shear rate scale by approximately the square of the inlet velocity. There were some differences near the front wall of the device, which was the major geometric difference between the two VAD designs. **Conclusions:** The local fluid dynamics plays a significant role in the development of thrombus on a surface. To facilitate the success of these VADs, measurements were taken for varying heart rates as would be seen clinically. A new wall shear rate correlation coefficient has been developed to correlate this variability to potential deposition.

O13 (EI0089)

Hemodynamics of a Valveless Counterpulsation Heart Assist Device: Laser Doppler Velocimetry and Computational Fluid Dynamics

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Objectives: Single port valveless pneumatic counterpulsations heart assist devices have had, normally, a high incidence of thrombus formation due to blood stagnation regions in the blood chamber. This prevents a success of these device types in a long-term application. Blood chamber flow of a novel 32 ml stroke volume blood chamber design was investigated. **Methods:** To investigate blood washout behavior of the new design of a valveless counterpulsation device (CPD) with disk-shaped blood chamber (30 mm radius and 25 mm maximal thickness), laser Doppler velocimetry (LDV) and Computational Fluid Dynamics (CFD) were applied. Simplified static CFD model using flow solver FLUENT (ANSYS Inc., USA) was used to visualize 3D flow structure at the end-filling phase. The time resolved flow investigation of the CPD chamber and the inlet port was done by two-component LDV device (Dantec Dynamics, Denmark). **Results and Discussion:** Flow investigations found that tangentially designed CPD inlet port of a 10 mm diameter forms during a filling phase a strong, in general two-dimensional (2D) moving vortex fully filling the blood chamber. Such vortex is considered to be indicative for a good washing. No regions of persistent blood stagnation or recirculation bubbles were observed. Laminar shear stresses estimated by CFD were well below the known hemolysis threshold of 400 Pa inside the blood chamber. The short curved graft generated the helical flow pattern forming a minor secondary flow (helicity) of the 2D vortex. This secondary flow is considered to be favorable for the **Conclusions:** The CPD blood chamber flow has good washing characteristics without stable areas of blood stagnation during the entire pump cycle thus promising a low risk of thrombus formations.

O14 (EI0085)

Comparison of the Flow Pattern and Stagnation Areas Development in the VAD under Transient and Steady State Conditions

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Objectives: A numerical flow analysis and a comparison of operation of the pneumatic VAD of the POLVAD EXT™ developed in FCS, Zabrze, type under steady state and transient conditions are discussed. In the transient test, a motion of the diaphragm as well as discs is simulated. The valves are based on J. Moll's design, with later modifications introduced at the Institute of Turbomachinery, TU Lodz. **Methods:** Flow simulations for two different approaches are compared. In the steady state, two opposite operational states, namely diastole and systole, are simulated. In order to fulfil code requirements in the steady state, both discs are open. For diastole, the inlet disc is fully open whereas the outlet one is almost closed. For systole, it is opposite. In the transient test, four cycles of operation of the VAD are calculated to minimize an influence of initial conditions on the analyzed flow. A motion of the diaphragm and discs are forced by equations developed on the basis of the VAD normal operation. Discs are modelled as immersed bodies and operate in a full range, from open to closed state with respect to the diaphragm motion. The non-Newtonian blood model based on the Power Law is applied. Velocity, streamlines, pressure distributions in the region of investigations are presented and analyzed. The ANSYS CFX v.12.1 code is used to perform the numerical experiment. **Results:** The numerical experiment conducted shows differences in flow patterns as the inertia of fluid particles plays a significant role in systole in the case of transient calculations. **Conclusions:** The numerical study shows that steady state simulations are useful in the pre-design stage as they need significantly smaller computational efforts. Nevertheless, the transient method with a moving membrane and discs allows one to identify flow structures that cannot be visualized in the steady state.

Ambulatory Blood Processing—Symposium

O15 (EI0435)

Design and in-vitro Performance of a Wearable Ultrafiltrator

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Objectives: Current ESRD therapy removes catabolites but is least effective in maintaining euvolemia. Control of hypo- and hypertension require frequent

water removal from ESRD and CHF patients. An ambulatory ultrafiltrator to address these problems that is safe, effective, and convenient is the object of this work. **Methods:** A two stage plasma filter—ultrafiltrator produces a plasma flow from unanticoagulated blood. It uses silicon microsieves and a micofluidic flow channel to achieve high plasma fluxes over a 30 cm^2 surface. The plasma stream is reduced in volume and returned to the patient. The device is intended to work continuously at 1 ml/min, equivalent to 10 kg/wk. **Results:** Transport feasibility and blood compatibility have been demonstrated in the laboratory. Animal experiments are underway. Specialized components including pumps and highly miniaturized sensors and monitoring devices have been developed. Fundamental information about microporous sieving of blood under controlled microfluidic conditions has been obtained, although the thrust of this report will be on the practical system. **Conclusions:** Feasibility of a wearable ultrafiltrator capable of supporting euvolemia in ESRD and CHF patients that is safe, effective and convenient has been developed to the point of advanced animal testing.

O16 (EI0335)

Blood Access for Wearable Devices: Catheters and Access Ports

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Objectives: Wearable devices for hemodialysis and ultrafiltration are currently developed employing readily available technology but also novel concepts for pumps, dialysers and dialysate regeneration. The problem of blood access for these devices has not been widely discussed so far. Patient questionnaires regarding acceptance of home hemodialysis show that patients are afraid of fistulas and grafts but also of catheters because of safety aspects and difficulties to self access and because of the fear of complications. This paper discusses possible blood access concepts for wearable devices. **Methods:** The investigation starts with the assumption that blood flows up to 100 mL/min are required to achieve a creatinine clearance of >30 mL/min which corresponds to kidney failure class 3 (moderate). For permanently connected wearable devices the long tunneled catheter may be the optimal blood access. For patients who want to disconnect temporarily as well as for home-hemodialysis patients implantable ports may be the better alternative. **Results:** Based on the experience with an implantable access port several years ago we have designed an access port system that incorporates the following features: Easy self-accessing with no bleeding or pain, fail safe flow shutoff in case of disconnection, novel transcutaneous tissue tract guide and infection prophylaxis when disconnected using an antimicrobial tixotropic gel. **Conclusions:** The envisaged blood access port will reduce risks and complications of blood access for wearable devices and home-hemodialysis patients considerably.

O17 (EI0400)

Nephron+ Wearable Artificial Kidney

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Objectives: Improving the health condition of renal patients via a wearable system for continuous blood purification. **Methods:** A wearable artificial kidney is being developed embedded in an ICT-environment for personalised use and remote surveillance and control.The wearable device makes use of nanomaterials for sorption filtering in combination with miniaturized sensors and actuators. This allows for a small and wearable device. **Results:** The basic design has been finished and the system is currently in the engineering phase. Some components and early prototypes have been tested, including first animal trials. **Conclusions:** The Nephron+ consortium is making rapid progress and is well underway to meet its goals.

O18 (EI0114)

Atp-Adenosine-Glutathione Cross-Linked Hemoglobin as Blood Substitute

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Objectives: Although Hb-based blood substitutes may offer a solution to transfusion medicine problems such as blood shortages, transmission of bloodborne pathogens and the RBC storage lesion, all commercialization attempts to date have been unsuccessful due to efficacy or toxicity issues. We have developed HemoTech, the next generation blood substitute that utilizes the concept of "pharmacologic cross-linking." **Methods:** HemoTech, which consists of bovine Hb cross-linked intramolecularly with *o*-ATP and intermolecularly with *o*-adenosine, and conjugated with reduced glutathione (GSH), has entered the regulatory process in the USA. Several mandated requirements have been met including viral and prion clearance validation studies performed by BioReliance (Rockville, MD, USA) and various nonclinical pharmacology, toxicology, genotoxicity and efficacy tests conducted at the Research Toxicology Centre (Pompezia, Italy). The effects of HemoTech on appropriate physiological measures in human cell systems, normal animals and disease models have also been determined. The clinical proof-of-concept was carried out by the Instituto Sierovaccinogeno Italiano (S. Antimo, Italy). **Results:** In this composition, while ATP prevents Hb dimerization, adenosine permits the formation of homogeneous polymers and counteracts the vasoconstrictive and pro-inflammatory properties of Hb via stimulation of adenosine A2 and A3 receptors. GSH introduces electronegative charge onto the Hb surface that blocks Hb's transglomerular and transendothelial passage and shields heme from nitric oxide and reactive oxygen species, thus enhancing vasodilation and lowering Hb's pro-oxidative potential. The results of preclinical and clinical studies indicate that HemoTech can work as a physiological oxygen carrier with prolonged intravascular persistence and produces no adverse nephrotoxic, neurotoxic, oxidative, or inflammatory reactions. It has vasodilatory activity and can reduce the vasoconstriction that follows hemorrhage as well as possesses high erythropoietic potential. **Conclusions:** The obtained results confirmed that "pharmacologic cross-linking" of Hb molecules with ATP, adenosine and GSH is highly effective in designing a viable blood substitute.

Animal Models for Tissue Engineering—Symposium

K3 (EI0423)

Animal Models for Osteo- and Chondroengineering

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It is important to test tissue engineering approaches before they are transferred to the clinical arena. In vivo studies are necessary and demanded by federal institutions. The studies have to be performed according to animal welfare guidelines causing as least suffering as possible. For bone regeneration, several models are available. First, a rat femoral drill hole model is applicable for screening purposes. Thereafter, the constructs can be applied in a rat nonunion femur defect model. The constructs are administered and tested by several imaging modalities. In the last phase, a sheep tibia segmental defect model with nail osteosynthesis can be used. The defect can be filled with constructs and healing can be assessed by µCT and histology. For chondroengineering a "humanized" nude mouse model is available. In the middle of the cartilage of human osteochondral discs, a defect is induced that can be filled with constructs. Subsequently, they are implanted subcutaneously in the back of nude mice. Thereby, an in vivo environment for the human construct is available. When constructs are proven to be effective in this model, a large animal model in minipigs can be employed. A chondral or osteochondral defect of different sizes can be made in the femur condyle of minipigs through mini arthrotomy. Subsequently, the defects can be treated with constructs. The faith of stem cells can be traced by a Xenogen camera. For this technique the cells are labelled with the luciferase gene. Upon injection of luciferin, photons can be visualized and measured by the Xenogen device. It is important to choose the optimal animal model for the hypothesis tested. It is also important to use sophisticated methods to obtain good quality data. Furthermore, one should have experience in applying the animal models to obtain consistent and reliable data.

O19 (EI0422)

Gene Expression and Cell Differentiation in Matrix-Associated Chondrocyte Transplantation Grafts: a Comparative Study

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Objective: Although scaffold composition and architecture are considered to be important parameters for tissue engineering, their influence on gene expression and cell differentiation is rarely investigated in scaffolds used for matrix-associated autologous chondrocyte transplantation (MACT). After testing the efficiency of cell-graft systems with very different scaffold characteristics for the treatment of cartilage defects in a horse model, we have comparatively analyzed the gene expression of important chondrogenic markers in four clinical applied transplant types. **Methods:** Residuals (n = 165) of four different transplant types (MACI, Hyalograft C, CaReS and Novocart 3D) were collected during surgery and analyzed for Col1, Col2, aggrecan, versican, MIA and IL-1β by real-time PCR. Scaffold and cell morphology were evaluated by histology and electron microscopy. **Results:** Despite the cultivation on 3D scaffolds, the cell differentiation on all transplant types didn't reach the levels of native cartilage. Gene expression highly differed between the transplant types. The highest differentiation of cells (Col2/Col1 ratio) was found in CaReS, followed by Novocart 3D, Hyalograft C and MACI. IL-1β expression also exhibited high differences between the scaffolds showing low expression

levels in Novocart 3D and CaReS and higher expression levels in MACI and Hyalograft C. **Conclusions:** Our data indicate that scaffold characteristics as well as culture conditions highly influence gene expression in cartilage transplants and that these parameters may have profound impact on the tissue regeneration after MACT.

O20 (EI0421)

Human Placental Alkaline Phosphatase Transgenic Animals as a New Tool for Tissue Engineering and Regenerative Medicine

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Mesenchymal stem cells differentiation capacity, production of cytokines, and immunosuppressive potential undoubtedly offer many therapeutic advantages. To further explore the therapeutic potential of regenerative treatments, it is necessary to trace the fate of individual donor or manipulated cells in the host organism. However, immune-mediated rejection of labeled cells is a general problem in transplantation studies using cells labeled with any immunogenic marker. Recently, we generated a novel *in vivo* cell tracking system consisting of a transgenic donor line ubiquitously expressing the heat stable enzyme human placental alkaline phosphatase (hPLAP). The corresponding transgenic recipient line expresses a heat labile mutant form of the enzyme ($hPLAP^{E429G}$). $hPLAP^{E429G}$ differs just in one amino acid from the wild type form. Due to the slight alteration hPLAP is not recognized as foreign by the immune system of the $hPLAP^{E429G}$ transgenic host. Nevertheless, the difference in heat resistance allows the identification of donor cells in histologic sections. To prove the utility of this system in regenerative medicine, we successfully isolated and characterized hPLAP-tg-MSCs from the bone marrow of hPLAP transgenic rats and mice. The cells were analyzed regarding the expression of stem cell surface markers, and differentiation potential. As a preliminary evaluation of their potentiality, we have evaluated their viability, proliferation and differentiation after seeding the cells in films, gels and scaffolding materials *in vitro*. The seeded cells on different biomaterials could be readily traced after 1, 3, 14 and 21 days of *in vitro* culture by hPLAP staining. As a proof-of-principle, we have injected bone marrow MSCs from hPLAP-tg rats *in vivo* into the knee joint of marker tolerant wild-type rats and found successful engraftment and differentiation of donor cells. In conclusion, this novel transgenic animal model may be a very useful tool to answer many open questions in MSC biology and regenerative medicine.

O21 (EI0424)

Host Response to Biomaterials Evaluated Through Different Implantation Models

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Objectives: The host response to a foreign body inherent to the biomaterials' implantation depends on both host and implanted material, particularly considering the host tissue diversity. The aim of this study was to compare the inflammatory response induced by implantation of starch-based scaffolds subcutaneous (SC) and intramuscular (IM) in rats. **Methods:** Two methodologies, wet spinning (WS) and fibre bonding (FB)), were used to prepare the scaffolds. The inflammatory response was assessed in male Sprague-Dawley rats (n = 2), weighting 380–400 g, 1 and 2 weeks post-implantation. In both models 4 scaffolds were implanted: SC through incisions in the dorsum and IM into the left and right scalenus dorsalis and gluteus muscles, respectively. The animals were kept in single-housed with food and water ad libitum and received analgesia in the first week. After each time period, each animal was anaesthetized with an intramuscular injection of ketamine/xylazine and sacrificed with an intracardial overdose. The scaffolds, surrounding tissue and nearby lymph nodes were explanted and used for histological analysis. **Results and Discussion:** The WS and FB SPCL scaffolds did not elicit extensive leukocyte recruitment in both subcutaneous and intramuscular implantations in rats. The subcutaneous implantation induced a slightly higher inflammatory response as compared to the intramuscular implantation. However, in both situations the nearby lymph nodes showed to be activated in the earlier stage, but less activated later in the implantation. Additionally, both WS- and FB-SPCL scaffolds showed to be well integrated in the host, independently of the site of implantation. **Conclusions:** The overall data suggests a good integration of the materials in the host, independently of the tissue location. The results showed that the SC implantation induced a slightly higher inflammatory response than the IM implantation with early activation of the lymph nodes. Nonetheless, a normal progress of the reaction was observed for all the conditions.

O22 (EI0306)

The Effect of the Differentiation Stage of Amniotic Fluid Stem Cells Seeded onto Biodegradable Scaffolds in the Regeneration of Nonunion Defects

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Objectives: Bone tissue engineering strategies mainly requires cells with high proliferative and osteogenic potential, and a suitable scaffold to support cellular development towards neobone formation. Amniotic fluid stem cells(AFSCs) have shown high self-renewal capability, and the potential to differentiate along the osteogenic lineage, while SPCL (blend of starch and poly(e-caprolactone)) fiber mesh scaffolds, developed by melt bonding, have shown promising results for bone applications.Therefore, in this study we have evaluated the functionality of SPCL scaffolds seeded with human AFSCs *in vitro* and *in vivo*. Furthermore, the influence of the differentiation stage of AFSCs on the regeneration of femoral nonunion defects was investigated in a nude rat model. **Methods:** AFSCs were seeded onto SPCL scaffolds and *in vitro* cultured for different periods of time in osteogenic medium in order to obtain: i) undifferentiated cells, ii) cells committed to the osteogenic phenotype and iii) "osteoblastic-like" cells. After these endpoints, cells were assessed and characterized for viability, osteogenic phenotype and matrix formation (ALP, SEM, immune fluorescence for collagen I, and calcium quantification assays). Afterwards, SPCL constructs with AFSCs at different stages of differentiation were implanted (4 or 16 weeks) for assessment of bone regeneration by m-CT analysis, and immune-histological characterization (osteocalcin, collagen I and VEGF). **Results:** AFSCs proliferated on the SPCL scaffolds, and showed a cellular commitment towards the osteogenic lineage after 2 weeks, with the production of a mineralized ECM after 3 weeks in osteogenic medium (osteoblast-like AFSCs). *In vivo* neoformation of bone was observed in all conditions. Nevertheless, the best bridging between the two sections of the defect was observed in the presence of SPCL scaffolds seeded with osteogenically committed AFSCs after 16 weeks. Blood vessels were also observed in the inner sections of constructs implanted with AFSCs. **Conclusions:** Results indicate that SPCL scaffolds combined with AFSCs evidence great potential of for bone regeneration in nonunion defects.

Cardiovascular General 2: Devices Interaction—General Session

O23 (EI0223)

Development of a Blood Pressure Simulator

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Objectives: The blood pressure simulator is needed for the development of a new method to measure blood pressure (BP) noninvasively over 24 hours. It has to provide a pressure curve similar to the blood pressure in the radial artery. In addition, it has to provide curves with added artefacts as they result from arm movement. **Methods:** The blood pressure simulator is driven by a linear motor acting on a piston. The piston is filled with air, which acts as a spring generating a pressure on an enclosed volume. The enclosed volume is filled with the model fluid, which is water containing polystyrene particles. They serve as reflectors for ultrasound waves, since the BP simulator is used for the development of a method using ultrasound to measure blood pressure noninvasively. A water column is connected to the volume generating a hydrostatic pressure p_{hydro}. The model fluid exits the blood pressure simulator and enters a model artery made of thin polyurethane foil embedded in a tissue model made of gelatine. **Results:** With this simulator any arbitrary pressure curve $p(t) = p_{hydro} + p'(t)$ can be generated in the artery model. The pressure fluctuation $p'(t)$ are generated by the piston movement $x(t)$ by a linear transfer function $p'(t) = k*x(t)$ with transfer coefficient k. The transfer coefficient depends on the piston area and the spring constant of the air spring. **Conclusions:** The blood pressure simulator is able to generate complicated pressure curves as they are found in the radial artery of a moving arm. It is such well suited for the development of a new method to measure blood pressure noninvasively.

O24 (EI0136)

Noninvasive Biological Parameters Measurement in Heart Prosthesis

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Objectives: For optimization, monitoring and partial-automation of mechanical heart support it is necessary to measure appropriate biological signals. The paper presents noninvasive measurement methods of those parameters. Goal of study was to construct and examine selected biological parameters measurement methods dedicated for pulsatile VAD. This work is a part of Polish Artificial Heart project. **Methods:** Following biophysical quantities' measurement methods were investigated: blood flow (ultrasound Doppler velocity profile methods), temporary blood volume in the pump (Helmholtz resonance and rheoimpedance methods), blood pressure (piezoresistive sensors separated from blood through the polyurethane membrane) and epicardial ECG. Investigations were performed on *in-vitro* models which simulated essential biophysical phenomena. Trial measurements were performed on animal (110 kg pig). **Results:** Accuracy of Doppler volumetric blood flow measurement was 20%. For rheoimpedance method difference between measured and reference volume (calculated by flow integration) was 0.44 mL with standard deviation = 3.5 mL (Bland-Altman plot). Helmholtz resonance method allowed to measure blood chamber volume with unreliability of 5%. Accuracy about 3 mm Hg and negligible hysteresis were obtained for blood pressure measurement gauge. QRS detector efficiency (estimated according to EN-60601-1-2-47 regulation) was >99%. **Conclusions:** Performed investigations allowed to select measurement methods appropriate to utilization in final construction of pulsatile VAD. Following methods were chosen: Doppler blood flow, pressure measurement by piezoresistive sensor and QRS detection from epicardial ECG. While developing VAD manufacturing technology the gauges constructions' limits should be taken into consideration.

O25 (EI0133)

Analysis of Cerebral Microembolism During Cardiopulmonary Bypass Dependent on Cannula Positioning: a Computational Study

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Objectives: Cerebral microembolism (CM) is a common problem in cardiopulmonary bypass (CPB) patients, resulting in postoperative neurologic malfunction. While the role of arterial line filters has been thoroughly studied, the possibilities to decrease CM by cannulation techniques have been neglected. In this study, a numerical model is presented to analyze behavior of clots in the blood flow dependent on cannula position. To achieve this, tracking of blood clots was implemented in a particle image velocimetry validated computational fluid dynamics (CFD) model. **Methods:** CFD simulations of CPB conditions with different cannula positions were performed in a 3D-model of the cardiovascular system which was derived from MRI data. Carotid and vertebral arteries were included to represent the cerebral vascular structure. 2000 clots with diameters of 100–500 micron were inserted through the cannula. The path of each clot was tracked to analyze conditions under which clots are washed into the brain. **Results:** The behavior of clots was affected by the positioning of the outflow cannula. A cannula tilt towards the cerebral vessels resulted in an increased likelihood for CM. In general, most clots reached the descending aorta and thus the peripheral vessels. Approximately 5% of clots below 200 micron and 2% of clots between 200 and 400 micron arrived at the outlets representing cerebral vessels, predominantly the carotid arteries. Less than 0.5% of larger clots were washed into the brain. **Conclusions:** This model provides the possibility to analyze different cannulation methods during CPB in terms of CM, allowing for better understanding of this phenomenon and thus better patient outcome. First results indicate that clots with smaller diameters are more likely to be washed into the brain. These clots have also a higher probability to escape arterial line filters. Simulations with different cannula designs and clot sizes below 100 micron are currently ongoing.

O26 (EI0123)

Effects of Pulsatile and Continuous Mechanical Circulatory Support on Regional Organ Flow. Experimental Study with Colored Microspheres.

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Objective: To measure the regional blood flow in different organs in healthy minipigs with a pulsatile VAD and a continuous centrifugal pump, first in conditions of total support and after in partial support conditions. **Materials and methods:** Eight healthy minipigs were used for this study. In four of them a Berlin Heart Excor VAD had been implanted (pulsatile flow) and in the other four a Biomedicus centrifugal pump had been used (continuous flow). In both cases the inflow cannula had been connected to the apex of the left ventricle and the outflow cannula anasthomosed to the ascending aorta. Once the pump is placed, a first (basal) injection of yellow microspheres in the left auricle is performed. Then the pump is started and working parameters adjusted to achieve the maximum pump flow (total support). These conditions were maintained during 30 minutes and after a second injection of eosin microspheres is performed. Then the pump flow was reduced to a half of the maximum flow (partial support) and maintained during the next 30 minutes, after that, a third injection of violet microspheres is performed. Finally the animal is sacrificed and samples of myocardium, kidneys, lung, liver, bowel, and brain were obtained to measure the regional blood flow during the experience. **Results:** During total support the flow in kidneys and lung increases to levels around 150% as compared with the basal flow either in pulsatile or in continuous groups. In partial support conditions the blood flow remains around the basal levels in both groups. In myocardium, liver, bowel and brain modifications in regional blood flow are not significant. **Conclusions:** Kidneys and lung have a different behavior in terms of regional blood flow during total support conditions as compared with myocardium and other organs studied despite of the flow pattern, pulsatile or continuous

O27 (EI0180)

Effect of Inflow Cannula on Rotary Blood Pump Speed Pulsing Strategy

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Objectives: Timing impeller speed change with respect to the native cardiac cycle to induce pulsatility in Rotary Blood Pumps(RBPs) applications could influence both ventricle volumes and heart unloading, and thus the potential for myocardial recovery. However, the physical properties of inflow cannulas (resistance, inertance, compliance) could also alter the result of these pulsations. The purpose of this study was to determine the appropriate physical properties of cannula to fulfill the desired effect of these pulsations on hemodynamic. **Methods:** A mock circulation loop was operated to produce left heart failure conditions. A mixed flow RBP, connecting from ventricle to aorta, was configured to alter rotational speed, with pulse peaks in systole or diastole. Tubings with different length, inner diameter and material were used as the inflow cannula. Pressure at both ends of cannulas, motor power, resulting hemodynamic measurements of ventricular and aortic pressure, VAD outflow, ventricular volumes, and stroke work were recorded. **Results:** For pulse peak in systole, pressure drops through inflow cannula were increased from ~2 mm Hg with 12 mm (inner diameter) tubing to ~5 mm Hg with 3/8″ tubing, with a phase delay of desired pulse peak increasing from ~0.028 s to ~0.056 s. 12 mm tubing with 2.33 times more than the original length resulted in further phase delay of pulse peak (~0.069 s), but a lower pressure drop (~4 mm Hg). Silicone 12 mm tubing produces a slight increase of pressure drop (~3 mm Hg), and a rise in phase delay of pulse peak (0.111 s), compared to PVC 12 mm tubing. The same trend was also found for pulse peak in diastole. **Conclusions:** Average hemodynamic pressure remained relatively unchanged for all four cannula tests. 12 mm PVC tubing with less than 150 mm in length proved the most compatible with pulsatile operation, by providing the lowest pressure drop, smallest phase shift and lowest motor power required for RBP pulsatile strategy.

O28 (EI0009)

A Venous Needle Dislodgement Methodology Based on Detection of Heart Pulses in the Extracorporeal Circuit

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Objectives: Accidental venous needle dislodgement (VND) during dialysis is a rare, but serious clinical event. Epidemiological studies, however, indicate that worldwide as many as 200 patients die each year from VND. Modern dialysis machines rely on conventional venous pressure monitoring (CVPM) to detect VND. However, better venous needle monitoring (VNM) is needed because in practice it is difficult to set alarm limits adequately to assure efficacy of VND detection at an acceptable level of false alarms. **Methods:** We have developed a new VNM method based on detection of heart pressure pulses passing from the patient via the blood access to pressure sensors in the extracorporeal circuit. An adaptive RLS algorithm is used on the pressure signals to eliminate the relatively large blood pump variations and extract the heart pulses. Disappearance of the heart pulses from the venous pressure indicates VND. Dislodgement is detected with a correlation between extracted venous and arterial heart pulses. High correlation is obtained if both needles are in place, and low correlation indicates dislodgement. **Results:** Ten HD treatments and 4 treatments with controlled VND were clinically evaluated. The venous needle was deliberately disconnected from the access during 30 s while the

arterial needle was still connected and the blood pump was still running. Both the ability to detect dislodgement and the robustness of the method with respect to false alarms were evaluated. The method was capable of detecting heart pressure pulses in all treatments. All VND events were successfully detected within 20 s after the needle was disconnected. In the majority of the treatments no false alarms were obtained, and in the remaining treatments the number of false alarms was less than with CVPM. **Conclusions:** The new VNM method is able to detect VND, and reduces the number of false alarms compared to CVPM.

Artificial Muscle for Internal Organ—Symposium

K4 (EI0363)

Artificial Muscle for the Actuator of the Artifical Internal Organs

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Objectives: For the development of the totally implantable artificial internal organs, size and weight are the important issue. Biometal, new shape memory alloy actuator had been used in the development of the various kinds of artificial internal organs such as artificial esophagus, artificial sphincter, and artificial myocardium. **Methods:** Biometal, Ni-Ti SMA, were used for the actuator in the various kinds of artificial internal organs. Crystal structure of the SMA was arranged by the nano technology, so, the durability and contractility of Biometal became useful. By the use of Biometal, peristalsis movement of artificial esophagus, closing function of the artificial sphincter, and contraction support mechanism of artificial myocardium were embodied. Using these systems, animal (goats) experiments were performed to confirm its performance, antithrombogenicity and durability by the use of the goats. **Results:** Satisfactory performance of the peristalsis movement of artificial esophagus, artificial sphincter, and artificial myocardium systems were observed. Performace and durability tests for artificial sphincter were performed and satisfactory results were observed over 3 months. Performace and durability of artificial myocardium system were evaluated in chronic animal experiments using goats in one month, however, electrical circuit was unfortunately broken in some system. **Conclusions:** Performance levels of Biometal were almost satisfactory in animal experiments, but durability of the total system must be discussed. These new artificial internal organ systems will be good news for patients in the near future.

O29 (EI0358)

Biomedical Engineering Approach for Mechanical Circulatory Assist Using Novel Shape Memory Alloy Fibres

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Objectives: We have been developing artificial myocardial and circulatory assist devices by using sophisticated Ni-Ti anisotropic shape memory alloy fiber (Biometal). Mechanical contraction in the single fibre actuator can be achieved by the application of electric current with simple controllers for the Joule heating, and the long-term durability has been tested for more than one billion cycles. In this paper, we presented the effects of cardiovascular assist devices on hemodynamics and preliminary results on the novel biomedical engineering approach for pediatric circulatory assist based on shape memory alloy fiber technology. **Methods:** We developed the myocardial assist devices for the left or right ventricular assistance along with the anatomically sustainable structure and cardiac functions. In vitro and in vivo experiments were performed for the examination of the hydrodynamic or hemodynamic functions. Thermodynamic properties in device were also examined during the assistance. **Results:** A single 100 micron fiber actuator unit exhibited approximately 0.4 kgf as exergy, which was to be used as work. Total weights of the artificial myocardium developed were less than 150 g including silicone covers. There were no significant size-related surgical complications around the organs by the installation of the device via left thoracotomy in animal experiments using goats. Pressure and flow increased by 5–10 % by mechanical contraction in each device application by using shape memory alloy fibre. **Conclusions:** Miniaturized artificial circulatory assist devices were useful for the mechanical circulatory assist. Moreover, the controllability of these devices might be effective for the immediate response on physiological circulatory demand.

O30 (EI0293)

Biomedal Muscles to Restore Contractile Function of Weak Heart

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Objectives: Existing VADs are single-ventricle pumps needing anticoagulation. We developed a biventricular external assist device that partially reproduces the physiological muscle function of the heart. This artificial muscle could wrap the heart and improve its contractile force. **Methods:** The device has a carbon fibre skeleton fitting a 30–40 kg patient's heart, to which a Nitinol based artificial muscle is connected. The artificial muscle wraps both ventricles. The Nitinol fibres are woven on a Kevlar mesh surrounding each ventricle. The fibres are electrically driven with a dedicated control unit developed for this purpose. We assessed hemodynamic performances of this device using a previously described dedicated bench test. Volume ejected and pressure gradient has been measured with afterload ranging from 10 to 50 mm Hg. **Results:** With an afterload of 50 mm Hg the system has an ejection fraction of 4% on the right side and 5% on the left side. The system is able to generate a systolic ejection of 2.2 ml on the right side and 3.25 ml on the left side. With an afterload of 25 mm Hg the results are reduced of about 20%. The activation frequency can reach 80/minute resulting in a total volume displacement of 176 ml/minute on the right side and 260 ml/minute on the left side. **Conclusions:** These preliminary studies confirmed the possibility of improving the ejection fraction of a failing heart using artificial muscle for external cardiac compression avoiding anticoagulation therapy. This device could be helpful in weaning cardio pulmonary bypass and/or for short-term cardio-circulatory support in pediatric population with cardiac failure.

O31 (EI0271)

Urinary Sphincter Based on Electronics and Artificial Muscles

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Objectives: The AMS 800 is the current artificial urinary sphincter (AUS) for incontinence due to intrinsic sphincter deficiency. Despite good clinical results, technical failures inherent to the hydraulic mechanism or urethral ischemic injury contribute to revisions up to 60%. We are developing an electronic AUS, called ARTUS to overcome the rigors of AMS. The objective of this study was to evaluate the technical efficacy and tissue tolerance of the ARTUS system in an animal model. **Methods:** The ARTUS is composed by three parts: the contractile unit, a series of rings and an integrated microprocessor. The contractile unit is made of Nitinol fibers. The rings are placed around the urethra to control the flow of urine by squeezing the urethra. They work in a sequential alternative mode and are controlled by a microprocessor. In the first phase a three-rings device was used while in the second phase a two-rings ARTUS was used. The device was implanted in 14 sheep divided in two groups of six and eight animals for study purpose. The first group aimed at bladder leak point pressure (BLPP) measurement and validation of the animal model; the second group aimed at verifying midterm tissue tolerance by explants at twelve weeks. General animal tolerance was also evaluated. **Results:** The ARTUS system implantation was uneventful. When the system was activated, the BLPP was measured at 1.038 ± 0.044 bar (mean ± SD). Urethral tissue analysis did not show significant morphological changes. No infection and no sign of discomfort were noted in animals at 12 weeks. **Conclusions:** The ARTUS proved to be effective in continence achievement in this study. Histological results support our idea that a sequential alternative mode can avoid urethral atrophy and ischemia. Further technical developments are needed to verify long-term outcome and permit human use.

Functionalized Biomaterials—Symposium

K5 (EI0444)

Functionalised Biomaterials for Organ Replacement and Regeneration

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Functionalized biomaterials are under development for medical devices and regenerative medicine to avoid side effects, to allow or inhibit the attachment of specific cells, to deliver drugs and mediators or to guide regeneration processes within the patient's body. This includes the development of new or modified materials with specific properties, e.g. materials offering niches for stem cells and influencing their differentiation or materials delivering mediators on a specific signal from the surrounding tissues or only in a defined cell type. The symposium will focus on some characteristics that can be achieved by designing appropriate biomaterials and on testing methods to prove functionality and safety.

O32 (EI0352)

Spider Silk Functionalized with Human Antimicrobial Peptides as a Novel Chimeric Protein for Tissue Engineering Applications

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Objectives: Genetically engineered fusion proteins offer potential as multifunctional biomaterials for medical use. Chimeric proteins can be formed using recombinant DNA technology by combining nucleotide sequences encoding different peptides or proteins that are otherwise not found together in nature. In the present study, three new fusion proteins were designed, cloned and expressed and assessed for function, by combining the consensus sequence for *Nephila clavipes* dragline spider silk (6mer) with three antimicrobial peptides: human neutrophil defensin 2 (HNP2), human neutrophil defensins 4 (HNP4) and hepcidin. Spider silk was selected for the core polymer due to its potential as a biomaterial to meet the requirements for both mechanical stability and biocompatibility, necessary for bone tissue engineering. The activities of the three different fusion proteins were compared to identify the most useful sequence for biomedical applications. **Methods:** The 6mer sequence was fused with the antimicrobial domains HNP2, HNP4 and hepcidin through step-by-step cloning. These proteins were assessed for their antimicrobial activity against *Escherichia coli* and *Staphylococcus* aureus through radial diffusion assay. Attenuated-total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was used to assess the secondary structure of the proteins. Cytotoxicity tests were performed to confirm the potential utility of these multifunctional silk proteins in contact with mammalian SaOs-2 cells. **Results:** Secondary structure analysis, performed by ATR-FTIR, indicated that silk maintains b-sheet formation capability even after adding the antimicrobial domains, which is good since is responsible for the exceptional mechanical properties of silk. Radial diffusion tests showed that the antimicrobial domains present in 6mer+HNP2, 6mer+HNP4 and 6mer+hepcidin proteins maintained bactericidal activity. Also, these proteins were capable of sustaining the proliferation of SaOs-2 cells. **Conclusions:** These new chimeric proteins suggest a new multifunctional approach to generate biomaterials with useful properties, in this case, control of infections due to the addition of the antimicrobial peptides.

O33 (EI0297)

Biomimetic Surface Modification on Artificial Hip Joint for Elongation of Implantation Life

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Objectives: According to anatomy of hip joint, articular cartilage surface is covered with hydrophilic natural polymers. This layer possesses water enough to enhance lubrication of bones. From the viewpoint of biomaterials design, recently, we have developed an artificial hip joint by using highly hydrophilic and biocompatible phospholipid polymer, poly(2-methacryloyloxyethyl phosphorylcholine) (poly(MPC)), grafted onto the cross-linked polyethylene (PE) surface. We hypothesize that the structure of surface-modified layers might affect the long-term stability, and the poly(MPC) grafted surface might assure the long-term performance of artificial joints. **Methods:** Grafting of the poly(MPC) on PE liner was carried out by photoinduced graft polymerization of MPC in aqueous medium. We examined that the effect of structure and performance of poly(MPC) graft layer on wearing of PE liner by hip joint simulator experiments. **Results:** The poly(MPC) grafting on the liners increased in hydrophilicity and decreased in friction coefficient, regardless of the cross-linking of the PE liner or the difference in the femoral head materials. During the hip joint simulator experiments (5×10^6 cycles of loading), the poly(MPC) grafting layer functioned well. That is, both decrease in gravimetric- and volumetric-wearing (i.e., particle production) were observed, while the femoral head materials did not affect it. The poly(MPC) grafting abrogated the wearing production, confirmed by almost no wearing of the liner surface, independently of the liner cross-linking or the femoral head materials. **Conclusions:** We concluded that the poly(MPC) grafting on the PE liner surpasses wearing effectively due to "fluid lubrication mechanism," that is as the same as the natural hip joint. Thus, extending longevity of artificial hip joints is expected. The technology has been applied for development of artificial hip joint and clinical evaluation in Japan has been finished.

O34 (EI0115)

Biomimetic Modification of Poss-Pcu Nanocomposite Using Response Surface Methodology

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Objectives: We have developed and patented a novel nanocomposite polymer for biomedical application, the material based on polyhedral oligomeric silsesquioxane (POSS) nanoparticle and poly (carbonate urea) urethane (PCU) polymer. It is widely accepted that biocompatibility depends on the surface properties of the biomaterial, and graft polymerisation is an attractive method to impart a variety of functional groups to it. Since the inert surface of POSS-PCU is not directly suitable for immobilisation of such biomolecules, therefore, graft polymerisation of acrylic acid (AA) and collagen over it was carried out (i.e. biomimetic modification). In this study, design of experiment methodology was also used to develop a predictive technique to optimize the operating conditions for grafting well controlled amounts of AA and collagen. **Methods:** Sheets of POSS-PCU were manufactured and the grafting of AA was carried out using a two-step plasma treatment (TSPT). The grafted films were characterised by ATR-FTIR, SEM, and water contact angle (WCA) measurements. The effects of two identified process variables (pretreatment and polymerisation time length); on the grafting density (GD) were investigated and optimized using central composite design in the response surface methodology. To test cell response to the protein gradient surfaces, human umbilical vein endothelial cells (HUVECs) were cultured on the substrates with different amount of bioactive components. **Results:** The presence of the AA grafted layers was confirmed by the appearance of a broad peak of the hydroxyl groups in ATR-FTIR spectrum, decreased in WCD, and morphological changes observed by SEM micrographs. It was found that the collagen was immobilised on the POSS-PCU surface with different amounts, and both the attachment and growth of HUVECs were dependent on the GD of it. **Conclusions:** These findings suggest that biomimetic modification of POSS-PCU could be an attractive way to improve blood compatibility and patency rate of small-diameter vascular grafts.

O35 (EI0050)

PH Variation During Layer by Layer Assemblies of Natural and Artificial Glycosaminoglycans to Control Cell Adhesion

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The study was aimed to develop biomimetic surfaces with possible biospecific cues to obtain control over cell adhesion by exploiting layer by layer (LBL) technique. Multilayers of heparin and synthetically made cellulose derivatives with heparinoid properties, prepared at different pH values were used as a tool to guide adhesion of skeletal muscle cells. LBL is a powerful technique based on alternating adsorption of oppositely charged polyelectrolytes (PEL) forms self-assembled nanostructures as multilayers. Application of biogenic PEL allows mimicking properties of the extracellular matrix. Heparin and cellulose sulfate (Cs1.94) as polyanions, while chitosan as polycation were used to prepare multilayers. The cellulose sulfate was applied as it is cost effective and highly bioactive regarding mitogenic and osteogenic activity. Variation in pH value of PEL was applied to control the physicochemical and biological properties of multilayers. Multilayers were characterized by water contact angle measurements (WCA), surface plasmon resonance (SPR), atomic force microscopy (AFM). Cellular investigations were done using C2C12 cell line. WCA measurements revealed that multilayer growth with alternating change in wettability by adsorption of different PEL is attributed to their functional groups. SPR also showed that mass of adsorbed material was dependent on pH value and type of adsorbed polyanion which was further confirmed by AFM measurements showing different topographies. A successful control on bioactivity of prepared surfaces was achieved by adjusting the pH value of polyanionic solutions. Cell experiments demonstrated that multilayers assembled at pH 9.0 were more adhesive for C2C12 cells than pH 4.0 layers and possible reasons for such behaviour could be given by wetting properties and charge of outermost layers pH variation leads to different multilayer properties. Multilayers prepared from artificial glycosaminoglycans like natural ones at specific pH conditions can be used as effective tool to obtain bioactive coatings on material surfaces that control cell adhesion.

Cardiovascular General 3: Physiology and Pump Control—General Session

O36 (EI0322)

Noninvasive Evaluation of Heart Rate Variability in Rotary Blood Pump Recipients Using Pump Data Only

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Objectives: In order to evaluate autonomic system function the heart rate variability (HRV) has been established as a valuable tool. In this study a method for a continuous monitoring of the heart rate and its variability in rotary blood pump recipients has been developed and evaluated. This method makes use of pump data only. **Methods:** Data from 10 rotary blood pump (RBP) recipients was analyzed. An algorithm was developed to estimate HRV parameters and to detect arrhythmic patterns from the pump flow signal. One hundred forty-seven datasets each of five minutes duration were analyzed including data recorded at the ICU, at the normal ward and during catheter-spiroergometry. Average heart rate (HR) and HRV parameters like the standard deviation of the NN intervals (SDNN), the square root of the mean squared difference of successive intervals (RMSSD), and the HRV triangular index (TI), were evaluated. Results were compared to those obtained using the ECG signal. **Results:** Medians and interquartile ranges of the HRV parameters calculated from the ECG were: HR: 98.8(89.6–117.1) bpm; SDNN: 17.9(7.1–38.1) ms; RMSSD: 11.9(8–42) ms; TI: 22.4(17–42.2) ms. Bland-Altman analysis showed that the HRV parameters derived from the pump flow were in close accordance with those derived from the ECG (mean ± SD of the difference in HR –0.3 ± 0.8 bpm; SSDN 1 ± 4 ms; RMSSD 3 ± 7 ms; TI 6 ± 12 ms). Arrhythmias like atrial fibrillation and extrasystolic beats could be detected as well using the proposed method. The accuracy of the HRV estimation method was not affected by changes in the hemdynamics, such as during exercise, Valsalva maneuvers or pump speed changes. **Conclusions:** Analysis of patient data showed that heart rate and its variability can be robustly detected from the pump flow signal, thus allowing a continuous monitoring of the patient autonomic system derangement and its eventual recovery.

O37 (EI0397)

Hemodynamics of Atrial Fibrillation in Rotary Blood Pump Recipients: a Simulation Study.

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Objectives: Atrial fibrillation (AF) is the most common cardiac arrhythmia and is frequently associated with chronic heart failure (CHF). 20–30% of patients classified in NYHA III-IV suffer from AF. In CHF patients with AF the cardiac output (CO) is usually reduced because of the missing "atrial kick" and of a concomitant mitral and tricuspid leakage. Aim of this simulation study was to investigate the hemodynamics of AF during rotary blood pump (RBP) support. **Methods:** A numerical model was employed to investigate the differences between normal sinus rhythm (NSR) and AF during RBP support at rest and during physical activity. The model was adapted to reproduce hemodynamics values derived from literature and from a RBP recipient suffering from AF undergoing a catheter-ergometry. Different pump speeds and changes in left ventricular contractility were simulated. **Results:** The CO of the RBP recipient suffering from AF is about 10% lower compared to the same heart condition and NSR; left atrial pressures (LAPs) are comparable. During physical activity the CO is also lower but with a much higher LAP (20%). The end systolic left ventricular volume (ESV) is reduced during AF, at higher pump speeds about 30%. In case of a recovering ventricle (improved contractility) and AF the CO increases but with a slight decrease of LAP only. **Conclusions:** Since RBP recipients suffering from AF exhibit a lower CO and an elevated LAP during physical activity a higher pump speed setting may be required compared to patients with NSR. On the other hand a higher unloading of the ventricle during AF results in a lower ESV, increasing the risk of suction. We conclude that the pump speed setting may be more crucial in RBP recipients suffering from AF than in patients with NSR.

O38 (EI0173)

Continuous Flow Left Ventricular Assist Devices Induce Left Ventricular Reverse Remodeling

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Objectives: Unloading of the left ventricle with pulsatile left ventricular assist devices (LVADs) induces reverse remodeling shown by a shift towards lower volumes of the passive end-diastolic pressure-volume relationship (passive EDPVR). Today continuous flow left ventricular assist devices are most frequently used for long term mechanical support. We addressed the question whether a continuous flow LVAD also induced a shift towards lower volumes of the passive EDPVR. **Methods:** All explants hearts from patients with chronic heart failure without an LVAD (=Group CHF, n = 5) and patients bridged to transplant with a continuous flow LVAD (HeartMate II, Thoractec corporation) (=Group Assist, n = 6) were prepared for measurement. A balloon was inserted inside the left ventricle through the mitral valve. Pressure in the balloon was measured during inflation of the balloon with incremental volumes of saline. Left ventricular capacitance was indexed by the volume at which the pressure reached 30 mm Hg (LVV_{30}). **Results:** Mean duration of mechanical support in the Group Assist was 366 ± 204 days. The LVV_{30} was significantly lower in patients with an LVAD (Group Assist: 139,4 ± 18,5 mL vs. Group CHF: 210 ± 56,2 mL; $p < 0,05$). Accordingly NT-proBNP levels at moment of transplantation were lower in the assisted group (Group Assist: 972,7 ± 675,8 ng/mL vs. Group CHF: 5838,7 ± 3546,6 ng/mL; $p < 0,05$). **Conclusions:** Reverse remodeling of the left ventricle is marked by a shift towards lower volumes of the passive EDPVR. This shift has been demonstrated in hearts unloaded with pulsatile LVADs. We demonstrated a same shift in hearts unloaded with continuous flow LVADs. Accordingly NT-proBNP levels were lower compared to unassisted chronic heart failure patients.

O39 (EI0244)

In Vivo Evaluation of a Hybrid Mock Circulation Loop Including a Baroreflex Model

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Objectives: A hybrid mock-circulation-loop (MCL) consisting of electrically controlled hydraulic elements and a software based model of the baroreflex autoregulation mechanism has been developed and constructed to evaluate control algorithms for mechanical circulatory support systems (MCS). The software detects an applied change in central venous pressure (CVP) and automatically adapts the MCL parameters to mimic the physiological response. The interaction of the developed software model and the MCL has been evaluated with in-vivo experimental data from animal trials. **Methods:** Two rotary blood pumps (MEDOS DP) were integrated in the MCL in a total artificial heart setup, pumping fluid through a systemic and pulmonary circuit. An electromechanically variable venous reservoir was added to the previously presented MCL, to accurately change the CVP. Hereby venous vasoconstriction can be mimicked. Upon a change in aortic pressure, the computer model automatically adjusts the MCL parameters peripheral resistance and unstressed volume as it is done by the autoregulatory system. For in-vivo validation, the native ventricles of the animal were replaced by the same rotary pumps and blood volume was varied by draining and infusing blood from and to the venous system. The baroreflex autoregulation response was measured through the changing MAoP. **Results:** For a change in CVP and a corresponding change in MAoP the model of the hybrid MCL replicates the baroreflex autoregulation mechanism and stabilizes the MAoP. The replicated response in the developed hybrid MCL and the in-vivo setting show a similar behaviour. With this new function of the hybrid MCL, a physiologic auto regulatory response to blood loss or a postural change can be simulated. **Conclusions:** The hybrid MCL can be used as an in-vitro tool to simulate physiological changes in the cardiovascular system. This facilitates the development of new control algorithms for mechanical circulatory support systems.

O40 (EI0100)

Video Evaluation of Kinematics and Dynamics of the Beating Cardiac Syncytium: an Alternative to the Langendorff's Method

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Objectives: Many important discoveries in heart physiology have been made possible using the isolated heart method of Langendorff. Nevertheless, this method has some limitations such as the vulnerability of the excised heart to injuries, the preconditioning during instrumentation, the possibility to induce tissue edema, and a high oxidative stress, leading to the deterioration of the contractile function. To avoid the preceding drawbacks, we have alternatively used beating mouse cardiac syncytia cultured *in vitro* in order to assess possible ergotropic, chronotropic, and inotropic effects of drugs. **Methods:** To achieve the preceding aim, we have developed a method based on image processing analysis to evaluate the kinematics and the dynamics of that drug-stimulated beating syncytia starting from the video registration of their contraction move-

ment. **Results:** In comparison with the physiological no-drug condition, we have observed progressive positive ergotropic, positive chronotropic, and positive inotropic effects of 10 µM isoproterenol (β-adrenergic agonist) and early positive ergotropic, negative chronotropic, and positive inotropic effects of 10 µM phenylephrine (α-adrenergic agonist), followed by a late phase with negative ergotropic, positive chronotropic, and negative inotropic trends. **Conclusions:** The present method permitted a systematic study of *in vitro* beating syncytia, producing results coherent with previous works. As consequence, it could be used in *in vitro* studies of beating cardiac patches, as alternative to the Langendorff's heart in biochemical and pharmacological studies, and, especially, when the Langendorff's technique is inapplicable (e.g., in studies about human cardiac syncytium in physiological and pathological conditions, patient-tailored therapeutics, and syncytium models derived from stem cells with genetic mutations). Furthermore, the method could help, in heart tissue engineering and bioartificial heart researches, to "engineer the heart piece by piece". In particular, the proposed method could be useful in the identification of a suitable cell source and in the development of "smart" biomaterials and novel bioreactors.

O41 (EI0036)

Safety Aware Pump-Control for a Rotary ECMO Blood Pump

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Objectives: During ECMO, about 12 % of all complications are caused by the blood pump or by air in the circuit. In addition, hemolysis, which bases on activation within the blood pump, causes complications during 7 % of all ECMO treatments. Since ECMO is still an ultima-ratio therapy we want to improve this situation by introducing a safe, but also automated ECMO setup, which can be run by a closed-loop control. **Methods:** We developed a remote steerable closed-loop control for a rotary blood pump. One of the major advantages of rotary blood pumps is a reduced activation of coagulation factors. To increase the safety of the blood pump operation, we introduced a model-based predictor. Thus, we are able to diagnose pump-related problems during ECMO like air in the circuit, suction of the cannulae or thrombi in the circuit. Air in the circuit and thrombi can be detected due to a behavior differing from the known pump characteristics. Suction of the cannulae is detected by blood flow and pressure gradients, and can be used for fault correction. **Results:** Applying closed-loop control, we were able to set a flow within a settling time of 1.08 sec and no overshoot. The introduced model-based supervisor enables us to detect discrete problems within the pump system. Thus, we can significantly enhance the safety of an ECMO treatment. **Conclusions:** The proposed pump control enables us to use a rotary blood pump as part of an automated ECMO setup. Due to the embedded safety models, we are able to decrease the risk related to an ECMO treatment. The introduced pump control is a next step closer to an automated ECMO setup. **Acknowledgements:** The authors gratefully acknowledge the contribution of the German Research Foundation DFG.

Vascular Access in Hemodialysis—Symposium

K6 (EI0313)

Hemodynamics in Vascular Access for Hemodialysis: an Overview

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Objectives: Chronic hemodialysis requires easy access to blood flow rates greater than those typically found in the extremities. The high flow, however, may lead to flow disturbances, responsible for the development of intimal hyperplasia, and possibly initiating stenosis formation and/or thrombosis. Therefore, many groups performed experimental as well as computational studies of vascular accesses in an effort to improve access outcome. **Methods:** An overview of our methods is presented. First, access models were manufactured using a transparent silicon elastomer to perform hemodynamic studies with a pulsatile flow. Second, access hemodynamics were also studied using Computational Fluid Dynamics. **Results:** The vascular access includes generally three regions of interest (ROI) regarding to the hemodynamics: ROI-1 is the arterial anastomosis and its importance for forearm ischemia/steal syndrome and intimal hyperplasia. Different studies were carried out. First, the shape of the arterial anastomosis was changed to investigate the flow pattern in a direct artery-graft connection and with a small diameter segment between artery and graft. And second, AV access-related ischemia was studied, by comparing experimentally different methods of treatment. ROI-2 is the artificial graft itself and the impact of graft length on the flow. And ROI-3 is the venous anastomosis and its trend to the development of intimal hyperplasia. The following venous anastomotic configurations were investigated: conventional end-to-side anastomosis, correctly trimmed Venaflo graft, untrimmed Venaflo graft, open side branch at the vein floor, subclavian loop graft with narrow versus wide inflow, anastomosis with an extreme stenosis at the vein floor, and additional disturbances of flow patterns within the venous anastomosis caused by single-needle hemodialysis. The results of these investigations are presented. **Conclusions:** The results here discussed refer to the clinical outcome, and are compared with the results of other selected study groups.

O42 (EI0188)

Development of a Capd Catheter with Infection Proof Exit-Site

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Objectives: In western countries peritoneal dialysis (PD) only plays a minor role compared to hemodialysis. This is partly due to the high risk of exit-site infections, the leading complications with peritoneal dialysis. The infection is caused by a biofilm, originating at the exit-site. Drug-eluting catheters or catheters containing silver particles have not yet solved the problem. Objective of the project is to develop an infection resistant catheter, which uses a mechanical method to permanently prevent the infection. **Methods:** The presented catheter is equipped with a protective sleeve, which surrounds the catheter in the skin penetrating area. Subcutaneously the sleeve is folded and the inner end is hermetically sealed to the catheter. It is made of medical grade polyurethane (PUR) and its surface is coated with polyethylene terephthalate (PET) fibers to enable the ingrowth of connective tissue. After the implantation the protective sleeve is slowly pulled outwards by means of a small traction device at a rate of few millimetres per week. Thus it can grow out of the skin but still moves fast enough to prevent the down growth of the biofilm. **Results:** In a key experiment 6 of the devices were implanted in goats. The catheters remained infection free over a period of 420 days, while control devices became infected. For the ongoing experiments the catheter setup was improved. So far 8 catheters have been implanted in 4 goats and show no signs of pocket generation. The experiment is designed to last one year. **Conclusions:** The newly developed catheters show good promise for the prevention of infections of the exit-sites of PD-catheters. The principle could also be used for other skin penetrating implants such as power lines of heart assist devices.

O43 (EI0185)

Clinical Evaluation of a New Type of Short-Term Hemodialysis Catheter With a Microdomain Surface

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Objectives: Short-term dual lumen catheters (below DLC) are a useful tool for vascular access in some patients but carry with it risk for complications due to thrombosis and infection. For this study, we utilized a DLC coated with a microdomain surface to improve anti-thrombogenicity and compared it with a regular non-coated DLC. **Methods:** For the comparison, we utilized the Gambro manufactured GamCath N catheter (below N) and compared it with the GamCath Dolphin catheter (below D). The D catheter has a very smooth surface designed to improve the anti-thrombogenicity of the catheter. We placed the N catheter 41 times and the D catheter 40 times to hemodialysis patients with maximal sterile barrier precautions. **Results:** The average age of the N group was 64 ± 17 and 71 ± 14 for the D group. For the majority of patients in both groups, the catheter was placed in the right jugular vein. Complications seen for the two groups were thrombosis in 8 patients (20%) in the N group and 2 patients (5%) in the D group. Infection was observed in 10 patients (24%) in the N group versus 5 patients (12.5%) in the D group. Duration of catheter placement was 15.2 ± 8.3 days versus 22.0 ± 10.1 days showing a statistical significant length of use ($p < 0.05$) for the D group. **Conclusions:** The new type of DLC with a microdomain surface showed a trend to decrease complications from thrombosis and infection, and showed promising results in terms of prolonging lifetime of the catheter.

O44 (EI0248)

Arteriovenous Fistula in the Elderly: Preoperative Ultrasonography Mapping and Construction by Interventional Nephrologists

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Objectives: The aim of our retrospective study was to evaluate ultrasonography preoperative mapping of vessels before the first attempt of constructing arteriovenous fistula (AVF) in patients aged ≥65 years. **Methods:** Two hundred four patients with end-stage renal disease (ESRD), aged 75 ± 6 years, 57% men and 40% diabetics, were included. Arteries and veins of both arms and forearms were examined by ultrasonography/Doppler preoperatively. Inner diameter of veins (under compression) and arteries were measured. Optimal and alternative positions for AV anastomosis were suggested. AVFs and grafts were constructed under local anesthesia, as outpatient procedure, all by

interventional nephrologist. **Results:** Adequate cephalic vein was present in 54% with diameter 4.9 ± 1.1 mm on right arm and in 59% with diameter 4.7 ± 1.2 mm on left arm. Suitable veins on forearm were recorded in 59% with diameter 3.6 ± 0.7 mm on the right, and in 55% with diameter 3.7 ± 0.8 mm on the left. Characteristics of arteries: diameter of cubital artery 4.5 ± 0.6 mm on the right and 4.5 ± 0.7 on the left. Diameter of right radial artery was 2.3 ± 0.4 mm, of the left 2.2 ± 0.5 mm. Diameter of right radial artery was measured less then 2.0 mm in 31%. On left forearm 37% of such arteries were found. In 76% (156/204) of patients AVF was constructed, with good immediate function in all but 4 patients, in 72% (113/156) on forearm and in 21% (33/156) on arm. Polytetrafluoroethylene (PTFE) grafts were created in 6% (10/156). In 24% (48/204) of patients construction of AVF/PTFE graft was not performed. AVFs were constructed with significant difference in females vs. males (68% vs. 83%), and in nondiabetics vs. diabetics (88% vs. 72%). **Conclusions:** Native arteriovenous fistula can be constructed by interventional nephrologists in the majority of elderly patients with ESRD. Diabetics and women were found to be worse candidates for construction of AVF than nondiabetics and men.

O45 (EI0139)

A Novel Method for Measuring Catheter Lock Spillage

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Objectives: Catheters are widely used for blood purification, parenteral nutrition and for the application of drugs. Previous work has focused on the theory and in-vitro demonstration of catheter lock spillage caused by the laminar flow profile and by fluid exchange caused by density differences. This work describes a method that potentially allows measurement of catheter lock spillage in-vivo without sampling. **Methods:** The method is based on the change of the electrical resistance of the catheter when the lock solution is injected. This method was tested in-vitro with 46.7% and 4% sodium citrate solution. The catheter tip was placed in a beaker filled with normal saline (46.7% citrate lock) and 5% NaCl (4% citrate lock). A stainless steal rod in the beaker served as one electrode and an Arrow-Johans ECG adapter (a luer connector with stainless steel electrode) which was placed on the distal end of the catheter served as second electrode. Conductivity was measured with a 5 V (rms) 310 Hz sinus voltage and a 10 kOhm resistor in series to the catheter. The driving voltage and the voltage drop at the catheter was continuously measured with a program written under LabView (National Instruments). **Results:** 47.6% citrate straight catheter: Catheter conductivity dropped following a single exponential curve. Catheter conductivity was reduced by 80% after 10 minutes and by 95% after 90 minutes. Curved catheter: The decay of the conductivity followed an exponential curve but the final value settled at approx. 50% of the difference compared to the straight catheter. 4% citrate (straight and curved): After injection of the lock the conductivity dropped but remained constant. The conductivity dropped further when a 10 fold volume was injected filling the catheter more completely. **Conclusions:** The data confirm previous results achieved with methods not applicable for in-vivo measurements.

Polymeric Membranes/Blood Interfaces—Symposium

K7 (EI0301)

Hemocompatibility Testing of Polymers

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Objectives: Biomaterials are commonly used in various chronic blood contacting applications such as prosthetic blood vessels or stents, etc. Surface properties of polymers (e.g. availability of functional groups, domain structure, electrical charge, hydrophobicity, interfacial adaptability, surface roughness) are assumed to determine the fate of blood proteins and platelets interacting with the materials. Until now, no consistent relationship has been found between hemocompatibility and these parameters. Because thrombogenicity represents the most common cause of graft failure and of stent thrombosis, polymers have to be tested for their thrombotic potential. **Methods:** Though the principles of hemocompatibility testing have been established (ISO-10993), they are to be seen as minimum requirements and supplementary tests need to be performed. But, because of the high variability of hemostasis a stringent test procedure and a very rigid stratification of the blood cell donors have to be applied. **Results:** Conditions for reproducible measurements will be shown and important pitfalls—that have to be avoided—will be discussed. Basically, there are two options to investigate the hemocompatibility polymers: static and dynamic setups. For static investigations, platelets are dropped onto the material surface and subsequently the adherence, activation, and spreading of the platelets is analyzed. To emulate dynamic testing conditions, closed-loop systems filled with platelet-rich plasma or whole blood are used. Due to the parabolic velocity profile, platelets float next to the tube wall so that interactions between platelets and the material under study is possible during circulation. **Conclusions:** Different factors interfere with hemocompatibility testing of polymers. A simple and clear-cut analysis is not yet developed so that a stringent test procedure has to be maintained and parameters according to the reaction of the platelets, the complement system, the plasmatic coagulation and the contact activation should be accounted for in the assessment of the hemocompatibility of polymers.

O46 (EI0405)

Polymeric Membranes with Tailored Barrier and Surface Properties

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Membrane technologies have been established in a wide range of industrial processes, and some medical applications are among the most successful examples. However, membranes can offer yet many more distinct advantages when applied as separator or contactor [1]. The majority of synthetic membranes are made from polymers because barrier and surface properties can be variied in wide ranges with help of established scalable manufacturing processes. Significant efforts have been made to further improve membrane performance by focussing on barrier properties (high selectivity, high flux) or surface properties (antifouling, biocompatibility). Important strategies include the development of novel membrane polymers which can form ordered self-assembled structures or membrane surface and pore functionalization with controlled functional macromolecular architectures. Membranes with "smart," i.e., stimuli-responsive barrier or surface properties, can also be created. All these developments had been critically reviewed [2–4]. Important trends which are most relevant for biomedical engineering will be discussed. Those will be illustrated with examples from own research, focussing on ultrafiltration membranes with pH- or temperature-responsive barrier properties, as well as membrane surfaces with minimized fouling, with high protein binding selectivity and capacity, or for recognition of specific cells. Finally, our contributions to an ongoing multi-partner project for the development of an *in vivo* biosensor ("diagnostic implant"), i.e., the designs of a polymeric hydrogel-based receptor/transducer element and of a blood-compatible polymeric barrier membrane, will be presented. [1] E. Drioli, L. Giorno (Ed.), *Membrane operations: Innovative separations and transformations*, Wiley–VCH, Weinheim, **2009**. [2] M. Ulbricht, *Polymer* **2006**, *47*, 2217. [3] D.M. He, H. Susanto, M. Ulbricht, *Prog. Polym. Sci.* **2009**, *34*, 62. [4] Q. Yang, N. Adrus, F. Tomicki, M. Ulbricht, *J. Mater. Chem.* **2011**, *21*, 2783.

O47 (EI0216)

Synthesis and Characterization of Bi-Soft Segment Poly (Ester Urethane Urea) (PEUU) Membranes for Extracorporeal Blood Oxygenation Devices

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Objectives: Polyurethanes are typically block copolymers that contain an ether/ester soft segment and an aromatic/aliphatic hard segment. The hard-to-soft segment ratio in polyurethanes can be changed by controlling the synthesis parameters in order to get tailored polyurethanes with improved physical and biocompatible properties. The structural versatility of polyurethanes with two soft segments has been subject of intense investigation in our group for the synthesis of symmetric and asymmetric gas permeable membranes. Having in mind the incorporation of these membranes in blood contacting devices and namely in membrane blood oxygenators, two main goals drove the synthesis of bi-soft segment asymmetric polyurethane membranes: i) The enhancement of O_2 and CO_2 gas permeation rates, and ii) The enhancement of the hemocompatibility. **Methods:** Tailoring of bi-soft segment asymmetric poly (ester urethane urea) (PEUU) membranes with enhanced hemocompatibility through the control of the surface morphology of the top dense layer. Bi-soft segment integrally skinned poly (ester urethane urea) (PEUU) membranes containing polypropylene oxide (PPO) and polycaprolactone (PCL) as soft segments are synthesized with PCL-diol ranging from 0%—15% (w/w). **Results:** The membrane with 15% (w/w) of PCL-diol shows the smoothest top dense layer with a R_a as low as 1 nm which is 5 times below the characteristic value of the PEUU membrane with a single soft segment. The PEUU 85 asymmetric membrane displayed minimal platelet deposition and inhibition of extreme stages of platelet activation. **Conclusions:** Atomic Force Microscopy characterized submicron roughnesses, R_a, of top dense surfaces of the asymmetric membranes as major assets to development of platelet/membrane surface interactions. Here we show that the top dense surfaces of asymmetric PEUU membranes can be tailored with different morphologies when the ratio of the two soft segments PPO/PCL varies. A strong correlation between the top surface roughnesses and platelet deposition is identified. **Acknowledgments:** The authors are grateful to "Fundação para a Ciência e Tecnologia (FCT)", Portugal for the financial help through contract programs PTDC/CTM/099595/2008 and REEQ1764/EQU/2005. Mónica Faria is grateful to INL—International Iberian Nanotechnology Laboratory for a PhD grant.

O48 (EI0073)

3D-Structuring of Poly(Vinyl Alcohol)-Based Photopolymers

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Objectives: The fabrication of 3D-scaffolds with defined pore geometries which enable good adhesion of cells is a challenging topic in the field of regenerative medicine. Photopolymers which can be structured by means of Additive Manufacturing Technologies are promising materials for this application. The possibility of structuring these compounds via processes such as microstereolithography (µSLA), Digital Light Processing (DLP) or Two Photon Polymerization (2PP) enables the fabrication of constructs with complex geometries and high resolution mimicking cellular structures of natural materials such as bone. **Methods:** Beside the considerable irritancy and sometimes toxicity of acrylate-based monomers, the formation of polyacrylic acid through hydrolytic degradation of the polymer is another undesirable aspect of these materials when applied in the biomedical field. Therefore, photopolymers with different polymerizable groups such as vinylesters, vinylcarbonates and vinylcarbamates which give water-soluble poly(vinyl alcohol) upon hydrolytic degradation, were evaluated. Several monomers were synthesized to examine the properties of these substance classes with focus on cytotoxicity, photoreactivity, mechanical properties and degradation behavior. 3D-parts made of the new materials were implanted into New Zealand White Rabbits to examine the behaviour under physiological conditions. **Results:** The biocompatibility of these new substances, measured by their cytotoxicity towards osteoblast-like cells, showed better results than for their (meth)acrylate-based counterparts. The photoreactivity was found to be between that of acrylates and methacrylates, mechanical properties were on the same level and degradation characteristics could be tailored over a broad range. The in-vivo studies showed excellent biocompatibility of the materials as well as osteoconductivity due to the layered structure inherent to parts structured with conventional AMTs. **Conclusions:** The prepared photopolymers based on poly(vinyl alcohol) show interesting properties for the application in the biomedical field. Under the maintenance of mechanical properties and photoreactivity of conventional photopolymerizable monomers based on (meth)acrylates, cytotoxicity and the degradation behaviour could be significantly improved.

O49 (EI0061)

Insights into the Role of Material Surface Topography and Wettability on Cell-Material Interactions

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Objectives: Effects of the nature of biomaterials and surface topography on protein adsorption, cell attachment, proliferation and morphology are evaluated and reveal important insights in the complexity of cell-material interactions. **Methods:** We investigated protein adhesion and C2C12 premyoblasts cell cultureon biomaterials of various wettability. Materials used were poly(dimethyl siloxane) (PDMS), poly(L-lactic acid) (PLLA), a co-polymer of poly(ethylene oxide) and poly(butylene terephtalate) (PEOT/PBT) and tissue culture polystyrene (TCPS). An micropatterned array of pillars with variable pillar spacing and pillar height was embedded on the biomaterial surfaces through solvent casting of the polymers in solution on specific molds. The morphology of the patterned materials was evaluated by scanning electron microscopy. **Results and discussion:** Our results reveal a clear effect of surface topography, and to a lesser extent of material hydrophobicity, on cell attachment, morphology and proliferation. Generally, surface topography on very hydrophobic materials improves initial C2C12 cell attachment, whereas less hydrophobic and nonpatterned materials seem to support higher cell proliferation and spreading. With respect to cell morphology, surface topography seems dominant over material wettability; though the transition where cells change from growing on top of the patterned pillars to growing on the underlying biomaterial surface appears to be determined by the material wettability. **Conclusions:** Surface topography, and to a lesser extent material hydrophobicity, has a clear effect on cell attachment, morphology and proliferation. These findings are important in the design of biomaterials in various applications including medical implants, bio-artificial organs and tissue engineering.

Nano- and Micro-Technology: Driving the Future of Organ Recovery & Development—Symposium

K8 (EI0434)

Microscale Technologies for Regeneration of Functional Tissues Models In Vitro

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Most tissues in organisms are composed of repeating cellular structures (i.e., functional units), such as the lobule in the liver and kidney, islets in the pancreas. *In vivo*, the cells in these functional units are imbedded in a three-dimensional (3D) microenvironment composed of extracellular matrix (ECM) and neighboring cells with defined spatial distribution. Tissue engineering approaches therefore attempt to recreate the native 3D architecture *in vitro*. Recently, the convergence of nano and microscale technologies and hydrogels has resulted in the emergence of bottom-up methods where cell-laden microgels can be used as building blocks for tissue engineering and regenerative medicine. Although various microgel fabrication and assembly methods have been developed based on modifying interfaces and using microfluidics, so far, two main challenges remain: (1) to fabricate microgels composed of multiple cell types spatially confined in 3D as functional units, and (2) to assemble microgels into large complex 3D constructs rapidly in an efficient way. We also developed a simple, non-invasive acoustic assembler for cell-encapsulating microgels with maintained cell viability. The microgels were assembled via acoustic field in seconds in a noninvasive manner. Besides, we developed novel cell printing technologies where microgel fabrication and assembly are integrated into one system. With cell printing, we have successfully regenerated muscle tissues, created *in vitro* cancer coculture models, and engineered controlled niches for embryoid stem cells. These methods that we present would enable a better biologically relevant *in vitro* platform to investigate cell–cell interactions in a 3D microenvironment, holding great potential in various areas, spanning tissue engineering, regenerative medicine, pharmacological studies and high throughput applications.

O50 (EI0415)

Enhanced In Vitro and In Vivo Osteogenesis of Rat Bone Marrow Stem Cells through Intracellular Delivery of Dexamethasone with Gelatin Micelles

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Objectives: Dexamethasone is known to promote osteogenic differentiation of stem cells. The objective of this work is to evaluate the effect of dexamethasone-loaded gelatin micelles and the consequent intracellular delivery on MC3T3-E1 (pre-osteoblastic cell line) and rat bone marrow stem cells (rBMSCs) in vitro and in vivo osteogenic differentiation. **Methods:** The internalization efficiency of the micelles was assessed by flow cytometric and confocal laser microscopic measurements after their in vitro culturing with the MC3T3-E1 and rBMSCs. Fluorescent-labeled micelles were added into the culture medium and the cell response was evaluated at different time points. The in vitro release of dexamethasone was also evaluated. The cells pre-incubated with the dexamethasone-loaded micelles, were then seeded in 3D gelatin hydrogels to evaluate the in vitro osteogenic differentiation. The hydrogels with cells were then implanted into a rat critical size bone defect and the bone formation was assessed by x-ray, micro computer tomography and histological analysis. **Results:** The internalization efficiency of the gelatin micelles by MC3T3-E1 and rBMSCs was higher than 90% for all the formulations. When cultured in vitro, the dexamethasone-loaded gelatin micelles enhanced the expression level of alkaline phosphatase and mineralization, as confirmed by higher calcium content and stronger alizarin red staining. When seeded into the 3D gelatin hydrogels, both types of cells pre-internalizing the micelles showed enhanced osteogenic differentiation compared with the nontreated cells. The rBMSCs pre-internalizing dexamethasone-loaded micelles and seeded into the 3D hydrogels showed enhanced in vivo new bone formation. **Conclusions:** The internalization of dexamethasone-loaded gelatin micelles by MC3T3-E1 and rBMSCs and the consequent intracellular delivery promoted the in vitro osteogenic differentiation and in vivo new bone formation. The present data suggest that the intracellular release of dexamethasone is a promising strategy of bone tissue engineering to improve the efficacy of osteogenic differentiation.

O51 (EI0134)

Investigation of a Computational Analysis for Gaseous Transfers between the Blood Layer and the Gas Layer in a Segment of a Hollow Fiber Bundle of an Oxygenator

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Objectives: We tried to estimate the gas transfer behaviors in a hollow fiber bundle of an oxygenator by a newly developed computational analysis method and compared its results with actually measured gas transfer performance. **Methods:** An invented numerical analysis method combines original programs that calculate membrane transfer source and blood-gas reaction source of both oxygen and carbon dioxide with commercialized computational fluid analysis software that can calculate mass transfer and fluid dynamics. The object oxygenator has a rectangular bundle consists of parallel and staggered arranged hollow fibers. Lengths of a segment model are 30 mm in the blood flow direction (full length of the bundle) and 4 mm in the gas flow direction (sufficient length for comparing with actual data). Velocity conditions were set up with assuming 1, 3 and 5 L/min of the blood flow rate and 1 of the ratio of gas to blood flow rate. Conditions of inflow blood were set at the AAMI values in both computational analysis and in-vitro experiment (PO_2: 37 mm Hg, PCO_2: 45 mm Hg and Hb: 12 g/dL). PO_2 and PCO_2 of ventilated gas were set at 713 mm Hg and 0 mm Hg. Results**:** The computational analysis demonstrated 72.5, 170.4, and 232.0 mL/min O_2 transfer rates and 66.1, 156.0, and 205.3 mL/min CO_2 transfer rates at 1, 3, 5 L/min blood and gas flows, respectively. In in-vitro experiment, O_2 transfer rates were 55.4, 153.1, and 229.4 mL/min and CO_2 transfer rates were 99.5, 117.5, and 151.9 mL/min at 1, 3, 5 L/min blood and gas flows, respectively. **Conclusions:** We conclude that newly developed computational analysis is a promising method for estimating gas transfer performance in an oxygenator, although further verification is necessary.

O52 (EI0346)

Functionalised Fibres for Sustained Delivery of Bioactive Substances via Emulsion Electrospinning

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Objectives: Functionalisation of electrospun fibres is one of the major challenges for their use as scaffolds in tissue engineering. Most biodegradable polymers require organic solvents for electrospinning, which typically denature the bioactive molecules. Hence, we describe here a modification to the traditional process, called emulsion electrospinning, to circumvent this problem. **Methods:** Poly(caprolactone) (PCL) was dissolved in chloroform at different concentrations, with Span 85 added as an emulsifier. The dispersed phase consisted of albumin and alkaline phosphatase (AP) dissolved in PBS. Alternatively, aqueous Eosin-Y solution was used for fluorescence microscopy. Stability of emulsions over time was studied using spectrophotometry. Fibre morphology was observed using scanning electron microscopy (SEM). For release kinetic studies, protein concentration was measured using Bradford assay. AP activity was measured using p-nitrophenyl phosphate as the substrate. **Results:** Stability of emulsions showed a distinct dependence on the polymer concentration in the solution with an optimum range between 15%—20 w/v PCL. SEM observations of fibre morphology also confirmed this range to be the most suitable for electrospinning. The proteins were released in a sustained manner from the fibres. AP released from the fibres retained up to 70% specific activity at day 3, indicating that the proteins did not denature during the process. **Conclusions:** A method was developed to produce functionalised polymer fibres using emulsion electrospinning. Best results were obtained with emulsions having PCL concentrations between 15% and 20%w/v. Using alkaline phosphatase, it was shown that the released enzyme was active and did not denature extensively during the process. Release of proteins over a longer period, as well as experiments to determine the cytocompatibility are currently in progress. This work is supported by funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for the Cluster of Excellence REBIRTH (From Regenerative Biology to Reconstructive Therapy).

O53 (EI0002)

Deposition Transfection Technology Using a Homopolymer with Both Thermoresponsive and Cationic Characters for Cardiac Gene Therapy

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Objectives: Effective cardiac gene therapy requires safe and effective gene delivery into the myocardium. Previously, we attempted deposition transfection technology for a novel nonviral gene transfection method, in which DNA complexes were kept in contact with their deposition surface using block copolymer with a cationic poly(*N,N*-dimethylaminopropyl acrylamide) chain and a thermoresponsive poly(*N*-isopropylacrylamide) chain. In this study, long poly(*N,N*-dimethylaminoethylmethacrylate) homopolymer with both thermoresponsive and cationic characters was developed as the material for deposition transfection method in cardiac gene therapy. **Methods and Results:** The polymer with molecular weight of 250 kDa was synthesized by photopolymerization. Complex formation between the polymer and plasmid DNA occurred immediately upon simple mixing in an aqueous medium; polyplexes ca. 40 nm in size were formed. Because the lower critical solution temperature of the polyplexes was approximately 32°C, they could deposit on the substrate by precipitation from an aqueous solution upon warming at 37°C, which was confirmed by x-ray photoelectron spectroscopy (XPS) for surface atomic analysis and water contact angle measurement. When Hela cells were cultured on the polyplexes-deposited substrate in a culture medium, the luciferase activity obtained was higher than that observed on a DNA-coated substrate and by conventional solution transfection using the polyplexes. By FACS measurement about 20% of cells were transfected for over 2 weeks with permissible cellular cytotoxicity. In vivo transfection activity of the system was investigated in rat. The polyplexes were injected directly into myocardium of rats and a higher level of Lac Z gene expression was obtained as well. **Conclusions:** A highly efficient novel nonviral vector based on deposition transfection technology using the thermoresponsive and cationic polymer was developed for myocardium of rats.

Roadbumps for Tissue Engineering Artificial Organs—Symposium

K9 (EI0442)

Current and Future Strategies to Address Manufacturing Challenges in Tissue and Organ Engineering

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Despite the compelling clinical need to regenerate damaged tissues/organs, impressive advances in the field of tissue and organ engineering have yet to result in viable engineered tissue products with widespread therapeutic adoption. The main challenges to be overcome have been identified in the yet not convincing benefit of the proposed therapies, combined with their high costs. Following the exemplifying paradigm of bone and cartilage regeneration, the lecture will highlight the bottlenecks of typical manufacturing strategies and will propose alternative bioreactor-based approaches for the manufacturing of 3D cellular grafts. The perspective will address issues related to quality standardization, process control and regulatory compliance in manufacturing cell-based products and highlight the need not only to automate, but also to streamline and simplify typical production processes. Examples will be given on the attractive paradigm to expand mesenchymal stem/progenitor cells from adult individuals directly in a "3D niche" environment, thereby maintaining a larger postexpansion differentiation capacity and bypassing the complex and costly serial cell passaging in monolayers. Finally, as a next generation paradigm, the lecture will propose and exemplify the concept of engineering regenerative strategies following principles of developmental biology, using the own body as the in vivo bioreactor.

K10 (EI0441)

Problems and Potential Solutions to Growth Factor Application in Tissue and Organ Engineering

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Most of the cells used for tissue and organ engineering (TE) need growth factors (GF) for survival proliferation and differentiation. While in vitro some GF can be part of the cell culture/perfusion medium, there is the necessity for local retention/application of GFs in vivo. Since those GF are typically needed over a longer period, a sustained (delayed) release or a local production is the goal. Therefore examples will be given for delayed release system by encapsulating and/or binding of GF to TE scaffolds. On the other hand local production will be discussed either from accessory cells (e.g. MSC) or from ex vivo transfected cells or from endogenous or applied cells, which are transfected in vivo. Different transfection systems will be shown with special emphasis gene activated matrices and on nonviral transfection systems. The latter technology holds great promise especially for using the body as its own bioreactor.

Artificial Liver—General Session

O54 (EI0420)

Imidazole Cyclodextrin (ImCD), Totally Synthetic Supramolecular Complex as an Antidote for Cyanide Poisoning

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Objectives: Cyanide (CN^-) poisoning has been recognized as one of the causes of premature deaths on building fire sites. Whereas sodium thiosulfate has been used to oxidize hemoglobin to methemoglobin to chelate CN^- ion, it is risky since it reduces O_2 carrying capacity of blood even less. We have synthesized imidazole cyclodextrin (ImCD), a totally synthetic supramolecular complex, which has extremely high affinity to CN^- ion. The purpose of this study is to evaluate the effect of ImCD for acute CN^- poisoning in cell culture *in vitro*, and the survival rate in mice as an antidote for CN^- *in vivo*. **Methods:** *In Vitro*: Murine 3T3 fibroblasts were incubated for 4 hours with 20% fetal bovine serum (FBS 100 µL, FBS group), 20%FBS and KCN 5 mM (KCN group), or various doses of ImCD (0.6–5.0 mM) and KCN 5 mM (ImCD group). Viability of the fibroblasts was determined by the activity of cytochrome-c-oxidase using MTT assay. *In Vivo*: Two-day survival rate was defined in BALB/c mice, pretreated with ImCD (0.023 mM/kg equi-molar to KCN) or saline, receiving KCN per oral at 0.023 mM/kg or LD_{100} dose. **Results:** The viability of incubated 3T3 fibroblasts, OD value in MTT assay, was significantly lower in the KCN group (0.027 ± 0.014) than in ImCD (5.0 mM) group (0.379 ± 0.010, $P < 0.001$), which was not significantly different from the value in the FBS group (0.380 ± 0.014, $P = 0.892$). While dose-dependent antagonistic effect of ImCD was demonstrated *in vitro*, mortality rate of the ImCD-treated mice (33%) was lower than that of saline-treated control mice *in vivo* (100%). **Conclusions:** The results suggest that ImCD has a potent antagonistic effect against CN^- cytotoxicity with a dose-dependent manner *in vitro*, and that ImCD has a possibility to reduce the mortality in CN^- poisoning *in vivo*.

O55 (EI0390)

Characterization of Different Activated Carbon Beads for Blood Purification: Adsorption Properties and Biocompatibility Analysis

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Objectives: A critical issue of the clinical syndrome in liver failure is the accumulation in the bloodstream of toxins not cleared by the failing liver. The removal of hydrophobic, albumin-bound substances can be extracorporeally achieved by artificial adsorption devices. Here, we report a study on the adsorptive capacity of CO_2 activated carbon spheres (average diameter 300 µm) with increasing activation levels (up to 86%). Additional analyses on the biocompatibility profile are presented. **Methods:** The adsorption properties of the carbon-based adsorbents were investigated in batch experiments through incubation with spiked human plasma under static conditions at defined time points. The adsorption of hydrophobic compounds accumulating in liver failure (i.e. bilirubin, cholic acid, phenol and tryptophan) as well as pro-inflammatory cytokines (TNF-α, IL-6) was assessed. Furthermore, both the activation of coagulation (monitored by intrinsic and extrinsic coagulation cascades) and the adsorption of individual factors were assessed. **Results:** The activation process results in the burning of amorphous carbons, leading to an increase in the specific surface area, the development of microporosity and a substantial shrinkage of carbon particles. Cytokines adsorption rose with increasing burn-off levels (39.2 pg TNF-α/mg adsorbent 86% activation and 6.3 pg TNF-α/mg adsorbent with 0% activation, after 60 min incubation). High adsorption efficiency of all the tested carbon beads for the removal of phenol, tryptophan and cholic acid was observed, however, the adsorption capacity for strongly albumin bound bilirubin did not increase significantly with progressive activation. No haemolysis could be detected for extracts of any of the tested adsorbents, and no abnormal time in the extrinsic and intrinsic coagulation pathways was recorded after incubation. **Conclusions:** Increasing burn-off levels enhanced the adsorptive performance of carbon particles, predominantly with respect of smaller adsorbates (IL-6, tryptophan, phenol, cholic acid).

O56 (EI0202)

Histomorphometric Analysis in the Progression of Hepatic Steatosis

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Objectives: The objective of this study is to create a method can be easily used to accurately determine the degree of steatosis through an analysis of histomorphometric images to provide a histological response objective. **Methods:** For this study were randomly enrolled 200 patients, including: 30 patients with normal histological examination performed for suspected disease, 170 patients with positive histology for different grades of steatosis. Two hundred images were analyzed for a total of 2275 sinusoids. The portions of each image occupied by sinusoids were outlined and the number of pixel of each sinusoid was calculated. The total area occupied by sinusoids within each specimen was then computed and reported. We have analyzed the form of sinusoids approaching this with an ellipse and then make the relationship between the two axes with the aim of measuring the hydraulic resistance. **Results:** We found a nonlinear progression in morphological and functional parameters between the different degrees of steatosis: hydraulic resistance, number of sinusoids [mm^2]; percentage of sinusoids in a frozen section, area of the sinusoids and form/area ratio of the sinusoids. **Conclusions:** This correlation between geometric analysis of images of individual histological changes of sinusoids could be predictive of the clinical evolution of disease. These results are not statistically significant, but suggestive to apply this methodology in a larger number of patients.

O57 (EI0200)

Delayed Graft Function in Liver Transplant. Role of Molecular Adsorbent Recirculating System

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Objectives: The aim of study is to highlight the capacity of albumin dialysis MARS to improve the clinical status in liver transplant patients affected by delayed graft function(DGF). **Methods:** From 1999, twenty seven patients affected by DGF were treated with MARS. Eighteen patients with similar MELD[20–25] score were enrolled in the study (Group A). The reference population of eighteen DGF (Group B) that showed similar MELD score was treated with Standard Medical Therapy (SMT). The number of MARS applications was $9 \pm 2,2$; the length of applications was 8 h. Serum values with relation to inflammation, excretory and detoxification function, synthesis capacity, renal function and hemodynamic parameters were measured using standard laboratory procedures before and at end of MARS treatments. The patients were valued for 30 days from inclusion with a survival follow-up at six months. **Results:** In patients treated with MARS therapy, all the variables detected in our analysis showed an evident improvement. The survival at 30 days and at three months was 100% and 95% respectively. Within three months, one patient underwent to liver transplant(LT). In Group B we obtained a survival at 30 days and at three months 88% and 61% respectively. Five patients underwent to LT. **Conclusions:** MARS therapy was able to improve clinical status and survival in patients with DGF. Moreover the use of MARS has avoided the retransplant of 89% patients. Further studies including larger numbers would be useful in confirming our findings.

O58 (EI0088)

Test Eaa for Endotoxin Activity in Acute on Chronic Liver Failure

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Objectives: The aim of our study is the making of early detection of endotoxin in patients with acute liver failure on chronic liver disease by the test of EAA and treated with Polymyxin-B hemoperfusion based (PMX-DHP) and Molecular Adsorbent Recirculating System (MARS) with follow up at 30 days. **Methods:** From 2008 to 2010, ten AoCLF patients with SIRS in association to suspect of infection and EAA-positive test (>0.60) were included in the study. These patients, in waiting list for liver transplant(LT), showed similar MELD score [range 19–25] and Encephalopathy Grade ≤2. Five patients received treatment to remove endotoxins with PMX-DHP and MARS treatment for liver failure(Group A). While the other five patients received MARS treatment only (Group B). **Results:** In Group A, two treatments PMX-DHP were performed on 4 patients (average EA = 0.66 [from 0.61 to 0.70]), three treatments for a patient (EA = 0.92) and all five patients underwent an average of four MARS treatments (range 3–5). At the end of therapy the median level of EA was 0.42 (range from 0.37 to 0.48). Measurements of lactates, IL-6, TNF-α were significantly improved in patients treated with these extracorporeal therapies. At 30 days from the observation, all five patients treated with MARS and PMX-DHP are alive. In Group B, a mean of 7.5 MARS treatments were performed. We observed an improvement in hemodynamic and liver functions with reduced levels of pro-inflammatory cytokines and lactates in 4 patients. One patient showed no improvement in clinical status with the development of sepsis and subsequent MOF after 24 days. **Conclusions:** The possibility of an early diagnosis with the EAA in AoCLF patients could prevent the progression of sepsis cascade. The use of PMX-DHP and MARS in these patients, could lead to resolution of clinical status in a short time.

O59 (EI0047)

Development of Material Systems for Engineering of Biohybrid Liver

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Objectives: Biohybrid liver represents a promising therapeutic system to sustain life of patients that suffer from acute hepatic failure but also in chronic liver diseases to bridge time to find a suitable transplant. One issue in biohybrid liver systems that has been rarely addressed is the development of membrane materials for the bioreactor that promote both hepatocyte attachment and intercellular contacts to maintain survival and function of cells. Here, specific polymers have been synthesized and also porosity of membranes has been adjusted to achieve this ambitious goal. **Methods:** Membranes were made from polyacrylonitrile or copolymers from acrylonitrile with N-vinylpyrrolidone, sodium methallylsulfonate or aminoethylmethacrylate by phase inversion. Porosity of membranes was in ultrafiltration range. One of the copolymers was also used to obtain a series of membrane materials with pore sizes ranging from 6 to 12 nm. Surface properties of membranes were studied with atomic force microscopy, water contact angles measurements and other methods. Biological studies were carried out in most cases with C3A hepatoblastoma cells but also with primary rat hepatocytes. **Results:** Copolymerization of acrylonitrile with the co-momoners resulted in membranes differing in wetting properties ranging from hydrophilic to moderate wettable materials. C3A hepatoblastoma cells grew particularly well on moderate wettable membranes having a more spread phenotype, while more hydrophilic membranes promoted intense cell-cell contacts with reduced growth but improved function in terms of cytochrome P450 activity. A change of porosity of hydrophilic membranes had also effects on growth and functional activity and could be used as additional tool to modulate hepatocyte behavior in the desired direction. **Conclusions:** Although promising effects of chemical and topographical properties of these novel polymer membranes on hepatocytes were shown and also protected by a variety of patents, none of the membranes has been used in commercial bioreactors so far, which will be discussed, too.

Tissue Engineering Approachs—Symposium

K11 (EI0437)

Thermo-Sensitive Hydrogel as Cell Carrier for Nucleus Pulposus Tissue Engineering

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Intervertebral disc degeneration usually starts at the nucleus pulposus. In the past decades, several techniques and prosthetics (artificial disc) have been developed to regenerate or replace the nucleus pulposus. However, these kind of pre-formed devices have to remove the nucleus pulposus and then replace an artificial one to relief the symptom of intervertebral disc degeneration. Recently, cell-based tissue engineering provides a rational approach to regenerate active nucleus pulposus cells (NP cells) to restore intervertebral disc architecture and function. However, the source of autologous nucleus pulposus cells are limited and their functional state does not favor regeneration. Besides, nucleus pulposus cells grown in monolayer may result in fibroblast-like transformation. Thus, the 3D hydrogel coculture system maybe an alternative method to provide an adequate environment for nucleus pulposus cells proliferation, extracellular matrix production, cytokines secretion. In this study, we demonstrated that cell proliferation, total DNA and sulfated glycosaminoglycans synthesis of nucleus pulposus cells were significantly increased in the 3D hydrogel coculture system. Furthermore, the extracellular matrix related gene expression and anabolism-related gene expression in 3D hydrogel coculture system were significantly higher than other culture condition (such as monolayer culture or cultured in 3D hydrogel without mesenchymal stem cells regulation).The gene expression of TIMP-1 and MMP-3 decreased in 3D hydrogel with mesenchymal stem cells coculture system. This study suggests that the thermo-sensitive hyrogel could be an adequate material for nucleus pulposus cells proliferation and extracellular matrix production. Moreover, mesenchymal stem cells could regulate the isolated nucleus pulposus cells back to normal state through paracrine communications in the developed 3D coculture system.

O60 (EI0417)

The Potential of Osteogenic Cell Sheets Cocultured With Endothelial Cells for Bone Tissue Engineering

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Objectives: Current strategies in the field of bone tissue engineering are limited by the use of scaffolds that present drawbacks such as cell necrosis at their bulk related to deficient vascularization after implantation. Cell sheet (CS) engineering has been proposed as a scaffold-free alternative for the regeneration of several tissues. This work proposes the use of this technology for bone regeneration by combining osteogenic CSs and endothelial cells. **Methods:** Osteogenic CSs were fabricated by culturing male rat bone marrow cells (rBMSCs) in thermo-responsive culture dishes in osteogenic medium. Human umbilical vein endothelial cells (HUVECs) were seeded on the rBMSCs to create cocultured CSs. The osteogenic CSs were recovered by lowering the temperature and then stacked on top of either a cocultured or a similar osteogenic CS, and transplanted to female nude mice. Implants were recovered after 7 days and characterized by hematoxylin&eosin (H&E) and alizarin red (AR) stainings, immunohistochemistry for osterix, osteopontin, SRY (to identify transplanted male rat cells) and CD31, and calcium quantification. **Results and Discussion:** H&E and AR stainings showed mineralized tissue formation in the implants both with and without HUVECs. Osterix and SRY immunostaining demonstrated the presence of host and donor osteogenic cells at the mineralization site showing recruitment of host osteogenic cells. HUVECs contribution to neo-vascularization was confirmed by identifying human CD31 cells in blood vessels. Furthermore, calcium quantification results showed a higher degree of mineralized tissue after the transplantation of the constructs with HUVECs. **Conclusions:** This work confirmed the potential of transplanted osteogenic cell sheets for bone regeneration as well as the advantage of promoting cross-talk between osteogenic and endothelial cells for improved new tissue formation. The proposed approach avoids the constraints of scaffold use while successfully addressing the important issue of implant vascularization.

O61 (EI0330)

Enabling Technologies for Organ Printing: Toward Organ Biofabrication Line

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Objectives: Organ printing is an emerging solid scaffold-free biofabrication technology or layer by layer additive bioprinting of functional 3D human tissue and organ constructs from self-assembling tissue spheroids. Bioprinter is a key tool for organ printing. It is becoming increasingly obvious that in order to bioprint human and organ constructs it is necessary to develop series of integrated automated robotic tools or an organ biofabrication line. **Methods:** The scalable technique for tissue spheroid biofabrication employs micromolded recessed template in nonadhesive agarose hydrogel, wherein the cell suspension automatically loaded into the template self-assemble into tissue spheroids due to gravitational forces. Robotic bioprinter for the precise dispensation of tissue spheroids include three essential elements: X-Y-Z axis robotic precision position system, three automated biomaterial dispensers (two aseptic valve sprayers and one automated tissue spheroids dispenser) and computer-based software enabled operational control. The first two dispensers spray sequentially fibrinogen and thrombin and enable instant biofabrication of thin layers of fibrin hydrogel, whereas another robotic dispenser punch tissue spheroids into sequentially sprayed layer of fibrin hydrogel. **Results:** It has been demonstrated that use of micomolded recession in on-adhesive hydrogel, combined with automated cell seeding, is a reliable method for robotic fabrication of uniform size tissue spheroids at large scale. It has been also shown that combination of hydrogel sprayers and tissue spheroids puncher enables to implement additive biofabrication of 3D tissue construct. The novel irrigation dripping tripled perfusion bioreactor with removable porous removable minitubes has been designed. Mathematical modeling and computer simulation demonstrated that proposed irrigation dripping circuit system will allow maintain viability of printed tissue constructs until the "build in" intra-organ branched vascular system will mature enough for initiation intravascular perfusion. **Conclusions:** Thus, presented data strongly indicate that design and development of fully integrated organ biofabrication line is challenging but achievable goal.

O62 (EI0395)

Microfluidic Encapsulation of Cells into Self-Assembling Xanthan-Phospolipid Amphiphile for Cell Therapy

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Objectives: We have investigated the synthesis of an amphiphilic polysaccharide, in which a phospholipid is attached to an anionic polysaccharide chain (xanthan gum), and its ability to self-assemble into capsular structures. Moreover, this work aimed to apply a microfluidic platform which can overcome inconveniences related with the heterogeneity of microcapsules produced by conventional systems. The properties and performance of the microcapsules were studied as well the ability of these self-assembled matrices to support the viability, function, and proliferation of encapsulated cells. **Methods:** Xanthan gum was conjugated with 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) using carbodiimide chemistry to activate carboxylic groups of xanthan and coupling to amine groups of DOPE. The polysaccharide-lipid amphiphile was characterized by physico-chemical methods, such as ^{1}H Nuclear Magnetic Resonance, Fourier Transform Infra Red spectroscopy, x-Ray Diffraction, Circular Dichroism and Scanning Electron Microscopy. A microfluidic device was used to fabricate microcapsules with controlled size and shape. ATDC5 cells (a murine chondrocytic cell line) were encapsulated within the microcapsules and their metabolic activity and viability were investigated. **Results:** The self-assembly of the amphiphilic polysaccharide in physiological ionic strength and pH resulted in the formation of stable hollow capsular structures. Using microfluidics, stable and homogenous microcapsules of xanthan-DOPE with average size around 300 µm were fabricated. ATDC5 cells were encapsulated within the capsules and remained viable and evidencing an increased cellular metabolic activity over 21 days of *in vitro* culture. **Conclusions:** By combining self-assembly of xanthan-DOPE and microfluidic microencaspsulation we were able to fabricate microcapsules that provided an adequate environment for cells to survive and proliferate.

O63 (EI0245)

Comparison of 3D static and Dynamic Cultures of Hepatocytes on Porous Pdlla

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Objectives: An hepatic bio-hybrid construct is device that marries hepatocytes to a scaffold able to guide cell migration, assembling and functionality. The construct should be able to mimic *in vitro* as much as possible a liver. Due to the difficulties in maintaining in the time viability and hepatic functions, culture conditions can deeply affect the construct growth. A dynamic cell culture system (i.e. a perfusion bioreactor) can be the necessary alternative to a static culture. The objective of this work was the comparison of long living and functional hepatocytes cell seeded PDLLA constructs cultured in static and dynamic conditions. **Methods:** Poly(D,L-lactic acid) (PDLLA) porous scaffolds were prepared by salt-leaching method. *In vitro* tests were performed on scaffolds seeded with mouse ANL12 hepatocytes cultured up to 14 days in static and dynamic conditions, by using a perfusion home-made bioreactor. Cell adhesion, proliferation and migration were characterized (Alamar Blue, SEM observation and Confocal Microscopy). Their functionality was evaluated by RT-PCR (Albumin, Plasminogen, EGF, TNF-alpha, Fibronectin). **Results:** Dynamic cell cultures showed better behavior with respect of the static conditions, both in terms of overall gene expression and 3D distribution. PDLLA scaffolds showed to be a good support for cell growth and proliferation, and the use of the dynamic bioreactor increased cell viability inside the scaffold thanks to a better diffusion of nutrients and oxygen inside the construct. **Conclusions:** The conclusion of this study confirmed the advantage of using dynamic cultures culture conditions to fabricate 3D hepatic cells seeded scaffolds that could be used for the in vitro toxicity assessment of drug or contaminants.

Cardiovascular General 4: Cardiopulmonary—General Session

O64 (EI0208)

Experimental and Numerical Results of a Neonatal Oxygenator with Integrated Pulsatile Pump and Heat Exchanger

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Objectives: Oxygenators are mostly used in combination with blood pumps and heat exchangers. To minimize surface area and priming volume of the extracorporeal circuit these components can be combined. This could reduce the risk of adverse reactions. **Methods:** Flexible silicone tubes (inner diameter 2 mm, wall thickness 150 µm) were placed symmetrically within the fiber bundle of an oxygenator. In combination with magnetic pinch valves at the oxygenators' in- and outlet these tubes generate a pulsatile flow through the oxygenator by collapsing and expanding. The pulsating tubes actively distribute blood inside the oxygenators' bundle. This could possibly increase the oxygenators' gas exchange efficiency as it reduces the risk of shunt flows and recirculation- or stagnation areas. Currently air is used to collapse and expand the tubes. By using tempered saline solution as driving fluid the tubes can be used as a heat exchanger also. **Results:** Five in-vitro tests were done following ISO 7199, using heparinized porcine blood. For flows up to 500 ml/min the oxygen and carbon dioxide transfer rates were 60–77 ml_{O2}/l_{blood} and 45–77 ml_{CO2}/l_{blood} respectively. The test modules priming volume was below 25 ml incl. the integrated pump. Numerical simulations using ANSYS CFD and in-vitro tests with a functional model prove the practicability of using the tubes as a heat exchanger. **Conclusions:** Integrating a pulsatile pump within an oxygenator by means of collapsing and expanding silicone tubes is feasible as the in-vitro tests show. The gas exchange was sufficient for the pumped blood flow in all five modules. Numerical and experimental results prove the practicability of using the silicone tubes in combination with tempered saline solution as a heat exchanger. This additional integration of a heat exchanger would further reduce the extracorporeal blood circuits' priming volume and potentially expand the range of applications for oxygenators.

O65 (EI0186)

Pulsatile or Continuous Cardiopulmonary Bypass: Infrared Thermography used to Settle This Dilemma

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Objectives: The debate about the possible advantages of pulsatile perfusion (PP) versus continuous perfusion (CP) in cardiopulmonary bypass (CPB) is still open. Aim of this study was to prove the suitability of infrared (IR) thermography as a new methodology to compare the effects on peripheral perfusion exerted by PP or CP. **Methods:** Patients (n = 10) undergoing aortic valve replacement were randomized in two groups subjected to either CP or PP. An IR thermocamera was used to acquire temperature maps on the sole of the patient's left foot during surgery. Five reference areas were identified on the sole and temperature vs. time tracings were analysed for each area. Continuous monitoring of the patient central (*i.e.* rectal and esophageal) temperatures and of the arterial and venous blood temperatures was performed using 4 temperature probes during the entire surgical procedure. The heating process at the end of the hypothermic surgical phase was analysed in terms of: time delay between the beginning of arterial blood rewarming and foot rewarming; slope of the temperature vs. time tracings during the heating phase and temperature differences among the five sole areas during the same heating phase. **Results:** PP patients showed more prompt answer of the sole of the foot to arterial rewarming (shorter time delay) with respect to CP. The slope of the temperature vs. time tracings during the heating phase showed to be steeper in PP than CP. A more homogeneous temperature distribution on the sole of the foot was obtained in PP patients than in CP. **Conclusions:** The IR thermography proved to be a suitable technique to evaluate peripheral perfusion during CPB. PP patients showed better peripheral perfusion than CP patients. This study is still ongoing, and a higher number of patients will be soon analysed to confirm the outcomes obtained so far.

O66 (EI0053)

Clinical Evaluation of New-Generation Oxygenators with Integrated Arterial-Line Filters for Cardiopulmonary Bypass

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Objectives: New-generation oxygenators with integrated arterial-line filters have been marketed to improve the efficacy of cardiopulmonary bypass (CPB). Differences in designs, materials, coating surfaces, pore size of arterial filter and static prime exist between the oxygenators. Despite abundant preclinical data, literature lacks clinical studies. **Methods:** From September 2010 to March 2011 80 consecutive patients undergoing aortic valve replacement were randomized to CPB using Terumo-CapioxFX25 (40 patients, Group-T) or Sorin-Synthesis (40 patients, Group-S) oxygenators. Pressure drop and gas-exchange efficacy were registered during CPB. Fluid balance, ACT, INR, aPTT, fibrinogen, platelets, serum-albumin, total proteins, white blood cells (WBC), high-sensitivity C-reactive protein (hsPCR) were measured after anesthetic induction (T0), at CPB-start (T1), before CPB-discontinuation(T2), at ITU-arrival (T3), 3 hours (T4) and 24 hours (T5) postoperatively. Clinical outcome was recorded. Repeated measure ANOVA and non-parametric statistics assessed between groups and during time differences. **Results:** The two groups showed similar baseline and intraoperative variables. No differences were recorded in pressure drop, gas exchange and acid balance (p = N.S. for all) during CPB. Despite similar fluid balance (between groups p = .979), Group-T showed lower serum albumin (between groups p = .014), total proteins (between groups p = .0001), fibrinogen (p ≤ .004 at T4 and T5), platelets (between groups p = .021), with higher INR (p ≤ .005 at T4 and T5), aPTT (between groups p = .0001), hsPCR (between groups p = .034), WBC (p = .003 at T5). Group-T also showed higher postoperative bleeding (p = .038) and need for transfusions (p = .0001). However, clinical outcome was comparable (p = N.S. for all clinical end points). **Conclusions:** Both oxygenators proved effective and resulted in comparable clinical outcome. However, Sorin-Synthesis seems to better preserve coagulative cascade and serum proteins, resulting in lower transfusions and post-CPB inflammatory response.

O67 (EI0054)

Selective Pulsatile Lung Perfusion with Oxygenated Blood in Low Risk Coronary Artery Bypass Patients Improves Pulmonary Haemodynamic and Respiratory Indices

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Objectives: Pulmonary dysfunction after cardiopulmonary bypass (CPB) still accounts for high morbidity and mortality. The aim of this prospective study was to evaluate the effect of pulsatile pulmonary perfusion (PPP) with oxygenated blood during CPB on postoperative lung function and overall outcome. **Methods:** Fifty low-risk CABG were prospectively randomized to receive PPP with oxygenated blood during CPB and aortic cross-clamping (25 patients, PPP-Group) or to conventional CPB (25 patients, control-Group). Pulmonary haemodynamic parameters [indexed pulmonary vascular resistances (PVRI), PAP, pulmonary capillary wedge pressure (WP), cardiac index(CI)] and respiratory indices [lung compliance (LC), PaO_2/FiO_2, alveolo-arterial-oxygen-gradient(A-aDO_2), mixed-venous-pO2(pvO_2)] were measured preoperatively, at ICU-arrival (T1), 3 hours postoperatively (T2), and postextubation (T3). Broncho-alveolar lavage fluid was collected preoperatively, at ICU-arrival (T1-BAL) and after 4 hours. Clinical outcome was recorded. **Results:** There were no differences in baseline variables and clinical outcome (p = NS for mortality, pulmonary morbidity, intubation time, ICU-stay). Patients undergoing PPP showed comparable pvO_2(p = NS at all time points) but better postoperative LC (T1: 72.6 ± 44.6 mL/cmH_2O vs 31.0 ± 6.9 control-Group:, p = .0001, T2: 78.9 ± 55.5 vs 33.4 ± 7.6, p = .0001, T3: 112.8 ± 43.3 vs 77.9 ± 26.1, p = .001), PaO_2/FiO_2 (T1:295.3 ± 22.3 vs 235.6 ± 72.4, p = .0001; T2:287.4 ± 47.3 vs 232.2 ± 32.4, p = .0001), with lower A-aDO_2 (T1:183.8 ± 25.5 mm/Hg vs 225.4 ± 61.1, p = .003; T2: 166.1 ± 41.7 vs 254.7 ± 33.3, p = .0001, T3: 96.0 ± 33.1 vs 124.8 ± 47.3, p = .016). CI (T1:3.1 ± 0.3 l/min/m^2 vs 1.7 ± 0.08, p = .0001; T2:3.3 ± 1.0 vs 2.1 ± 0.4, p = .0001; T3:3.6 ± 0.6 vs 2.5 ± 0.5, p = .0001), PVRI (T1: 296.8 ± 99.8 dyne·sec·m^2/cm^5 vs 551.6 ± 61.5, p = .0001, T2: 243.9 ± 58.3 vs 312.0 ± 23.6, p = .0001, T3:251.9 ± 42.8 vs 295.9 ± 27.8, p = .0001), WP (T1:8.4 ± 3.3 mm/Hg vs 15.8 ± 4.7, p = .0001; T2:7.8 ± 1.9 vs 10.4 ± 2.2, p = .0001) and PAP (T1:19.2 ± 3.3 mm/Hg vs 27.4 ± 4.5 p = .0001; T2:19.9 ± 3.3 vs 25.4 ± 2.5, p = .0001; T3: 16.5 ± 3.1 vs 21.0 ± 1.9, p = .0001) proved better in PPP-group. T1-BAL demonstrated lower neutrophils (79.3 ± 3.8% vs 85.0 ± 4.1%,p = .0001) and higher monocites (15.0 ± 7.2% vs 10.1 ± 1.4%,p = .001) in PPP-group. **Conclusions:** PPP with oxygenated blood during CPB does not impact clinical outcome but significantly improves pulmonary haemodynamic parameters and respiratory indices in low-risk CABG.

O68 (EI0103)

Physiological Target Control in Long Term Extracorporeal Oxygenation

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Objectives: The application of long-term extracorporeal lung assist is still limited in clinical practice. Apart from long term material issues of oxygenator and blood pump also the usage scenario itself poses major implications on the design of the whole treatment system. While in the OR such machines are continuously operated by dedicated personnel, in the ICU operation and supervision is only intermittent or triggered by alarms. Especially the "easy" task of setting the appropriate machine parameters requires continuous readjustment of machine operating values to patient demand and condition. In order to achieve this, we developed a closed-loop control system which enables the direct control of physiological target values. **Methods:** A fully computer controllable ECLA system featuring a centrifugal blood pump and a electronic fresh gas mixer was set up. Additional measurement equipment for blood flow and on line blood gases was integrated into the external circuit and supplemented by an extended patient monitoring. A cascaded control scheme was developed and implemented on a DSPACE real time control system. The inner control loop is used to control the oxygenator output gas concentrations. This enables the independent control of O_2 and CO_2 gas transfer. The outer control loop then uses this to control physiological target values of venous CO_2 and arterial O_2 saturation. **Results:** The control scheme was tested in an animal trial study of 8 pigs. By applying hypoxic gas concentrations at insufficient levels of minute ventilation, lung failure was simulated. Our physiologic control was tested at different levels of simulated lung failure. During 90% of the time target values could be kept within close boundaries. **Conclusions:** Physiological target control is one of the key issues in treating lung failure with long-term ECLA. We could show the feasibility of our approach and its robustness against disturbances.

O69 (EI0005)

Heart Lung Machine and Extracorporeal Life Support: A Modular System

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Objectives: Today, devices for extracorporeal circulation (ECC) like oxygenators and heat exchangers are limited to few different sizes. A total system replacement is performed for troubleshooting device complications as well as for switching from Heart Lung Machine (HLM) to Extracorporeal Life Support (ECLS), even though the components are partially identical (blood pump, oxygenator, heat exchanger). Therefore, we follow an entirely new concept, designing a modular system for: **1.** An ECC adaptable to different patient sizes from neonate to adult. **2.** A component-wise module exchange after complications. **3.** The ability to switch from HLM to ECLS without total system replacement. **Methods:** The system is modular regarding heat exchanger and oxygenator. It is adaptable to patient size and ECLS therapy by the number of modules. The pumps´ blood flow is divided into the active number of parallel lines containing oxygenator modules and if required heat exchanger modules. A test circuit was set up for hydrodynamic measurements of pressure drop over the separated oxygenator and heat exchanger modules and of flow deviation to three parallel lines. **Results:** Results of the hydrodynamic measurements show a total pressure drop over an oxygenator module and a heat exchanger module similar to a conventional oxygenator with integrated heat exchanger. The flow measurements demonstrate an equal flow through each parallel line. **Conclusions:** The hydrodynamic measurements show the feasibility of a passive uniform flow deviation to parallel lines with heat exchanger and oxygenator modules. The pressure drop over the modules confirms the modular design concept. Currently, we are designing a first laboratory model of the ECC-system containing: inflow cannula, venous reservoir, rotary blood pump, several heat exchanger and oxygenator modules and an outflow cannula. Blood tests will be performed for the proof of concept.

Artificial Kidney Dialysis—Symposium

K12 (EI0051)

Dialyzer Inlet Pressure Is a Late Indicator for Filter Clotting During Hemodialysis

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Objectives: A possible way to recognize thrombus formation within the capillaries of the hemodialyzer is to monitor the flow resistance by means of pressure measurements at the dialyzer inlet. When the dialyzer inlet pressure is measured continuously, trends of increasing flow resistance due to capillary occlusion could be predictive for developing coagulation. The purpose of this study is to assess the predictive value of dialyzer inlet pressure monitoring with regard to hemodialyzer clotting. **Methods:** Dialyzer inlet pressures were measured in-vitro during simulated hemodialysis treatments on a Fresenius 4008 with additional dialyzer inlet pressure sensor. During treatment the effective surface area of the capillary bundle facing the blood flow was varied by placing PVC rings of different sizes between the arterial hemodialyzer cap and the surface of capillary bundle. The arterial bundle surface area was varied between 0 and 90% in steps of 10%, and the corresponding increase in dialyzer inlet pressure was monitored. **Results and Discussion:** According to the law of Hagen-Poiseuille, any increase in flow resistance in a bundle of cylindrical tubular hemodialysis capillaries should be mirrored by a pressure increase if blood viscosity and flow are constant. However, pressure increase in the post-pump arterial tube segment was below 5% for a surface area reduction of the hemodialyzer below 50%. A significant increase in dialyzer inlet pressure was only found if already 80% of all filter capillaries were blocked. **Conclusions:** In principle, monitoring of dialyzer inlet pressures during therapy is a simple tool to identify patients at risk for thrombus formation. However, be aware that if you find a distinct pressure increase in the post pump arterial pressure segment, the filter has already gone most of its way to thrombosis. It follows that dialyzer inlet pressure is a late predictor for filter clotting.

O70 (EI0349)

What Are Possible Reasons for Inflammatory Reactions Observed in Peritoneal Dialysis?

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Aim of the study: Fluids for peritoneal dialysis may induce adverse clinical effects as reported in the literature. We tested the hypothesis whether complement activation induced by polysaccharides through their hydroxyl moieties may contribute to these effects. **Methods:** Six polysaccharides of different molecular weight were analyzed for their complement activating potential. Polymeric sugars, such as inulin, starch and Gluco-Pyranose-Polymer (Icodextrin), oligomeric sugars, such glycogen, and dimeric sugars, such as maltose and sucrose were used. 1. Polysaccharides were dissolved in saline and the resulting solution submitted to complement assays. 2. Polysaccharides were immobilized as adducts to activated human serum albumin at the surface of microtiter plates in order to simulate the adsorption of sugars to a solid surface, such as the peritoneal membrane.Complement containing serum was added to the prepared sugar solutions or incubated together with albumin linked sugars immobilized on MTPs. After incubation of the MTP at 37°C (1 hour) aliquots were collected and stored at 4°C. The formation of the stable split product C3a-desArg was assessed with the MicroVue Enzyme Immunoassay in triplets of aliquots. **Results:** Complement activation is found with soluble as well as with immobilized polysaccharides. The C3a increment ranged from 12–60 µg C3a/ml. C3a-generation is found in similar patterns in both experimental series. Both, biomaterials and polymers bearing hydroxylgroups are known to stimulate the activation of the complement system. Our investigations show that polymeric, oligomeric and dimeric polysaccharides are able to do so independent of whether they are dissolved or immobilized at a solid phase. Thus, all polysaccharides used in PD as osmotic agents may be involved in the stimulation of the immune system. **Conclusion:** Evidence is provided that therapeutically used polysaccharides may induce the activation of the alternative complement pathway and may contribute to aseptic inflammatory processes seen in PD.

O71 (EI0206)

Improving Hemodialysis Adequacy Outcomes through Cannulation Planning by Numerical Simulation

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Objectives: Cannulation is a key feature in hemodialysis as the hemodialyzer, but this procedure is much more empirical. Permanent access to an adequate blood flow is a requirement for successful hemodialysis session, where as repeated cannulation can damage the arteriovenous fistula (AVF). In this study, we propose to numerically model the blood flow in a side-to-end functional AVF and compare retrograde versus antegrade cannulation. We assess the hemodialysis adequacy by varying the needle position and orientation. **Methods:** In a previous work we published a 3D realistic numerical simulation of flow patterns and wall shear stresses of a patient specific AVF. In this study we added two back-eye needles to the venous site. A nephrology nurse prescribed a cannulation configuration, the needle position and orientation. Then we varied both parameters for the purpose of comparison. ICEM CFD and ANSYS CFX are used to mesh and solve the governing equations respectively. **Results:** Numerical results showed abnormal blood circulation in the venous part of the AVF, caused by recirculation flow and low wall shear. Visualizing the blood flow streamlines with the needles revealed that particles from the arterial needle slipped back to the venous needle. Placing a needle against the blood flow exerted high venous pressure and possibly damaging the blood cells. **Conclusions:** Proper cannulation of the AVF is essential to deliver adequate hemodialysis. There is disagreement whether needle orientation is or not associated with recirculation and/or in elevated venous pressure. This tool can be used in clinical practice for cannulation planning.

O72 (EI0107)

Safety and Efficacy of High Cut-Off Haemodialysis in Chronic Dialysis Patients: a Randomised Controlled Trial

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Objectives: High cut-off (HCO) haemodialysers are a new generation of dialysers with increased membrane permeability offering increased permeability to middle-molecules. We assessed thesafety and efficacy of their use in a chronic dialysis population **Methods:** Twenty-nine prevalent stable haemodialysis patients were randomised to two groups (A/B) in a cross-over study: (A) received one HCO-HD (Gambro HCO 1100) and two standard HD sessions (Polyflux 170H) per week for 8 weeks, followed by 3x HCO-HD for 8 weeks; (B) received these treatments in reverse. Safety was assessed by serum albumin levels and adverse events. Patients were withdrawn if serum albumin dropped by >25% or a thrombotic event occurred. Efficacy was assessed by dialysis dose (Kt/V), removal of middle molecules, phagocytic functional test and pulse wave velocity. **Results:** HCO-HD use thrice weekly caused a significant decrease in albumin (40.6 g/L ± 3.4 to 36.7 ± 2.6; $p < 0.01$) over 4 weeks, however no further reduction was observed by week 8 (37.4 ± 3.4), and no significant change occurred with HCO-HD used once weekly. No patients were excluded from the study for albumin loss. Four patients experienced adverse events; (x1) under-dialysed, (x3) clotted AVF. Mean Kt/V for HCO-HD was decreased compared to standard dialysis: 1.03 ± 0.17 and 1.31 ± 0.21 ($p < 0.05$). Pulse wave velocity was reduced during thrice weekly treatment. Polyclonal FLC concentrations were significantly decreased in patients receiving HCO-HD thrice weekly (median decrease 18.17% (–139.20–56.47)) compared to once weekly (median decrease –5.50% (–85.76–14.24); $p = 0.008$). Phagocyte function increased over the thrice weekly period from 91.2% ± 9.4 to 98.1% ± 2.1 ($p = 0.02$). **Conclusions:** HCO-HD offers clinicians the possibility for increased removal of larger uraemic toxins. Importantly, albumin loss plateaus by week 4 and was tolerated by all patients. Further work now needs to be undertaken assessing larger surface area HCO-dialysers and clinical surrogates should be studied in a larger population.

O73 (EI0062)

In Spite of Positive Charge on Polyethyleneimine, An69 St Membrane Does Not Tightly Adsorb Heparin During Continuous Renal Replacement Therapy

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Objectives: We compared the effects of priming with different heparin doses (5000 IU/L in group A vs. 20000 IU/L in group B) on filter life span and systemic coagulation parameters in critically ill patients with acute kidney injury in this randomized crossover study. **Methods:** Different doses of heparin were randomly assigned to 24 patients (M:F = 18:6, median of age 68 (range, 50~96 years)) at the 1st and 2nd filter during renal replacement therapy (RRT). **Results:** There was no difference of median values in baseline hemoglobin (9.1 (7.1~13) g/dL vs. 9.4 (8.3~11.2) g/dL, p = NS), platelet count (110500 (37000~352000)/mm³ vs. 108500 (23000~281000)/mm³, p = NS), activated partial thromboplastin time (aPTT, 37.9 (27.2~180) sec vs. 35.9 (27.2~97.2) sec, p = NS), prothrombin time (PT, 1.26 (0.98~2.09) INR vs. 1.19 (0.97~1.86) INR, p = NS), collagen/epinephrine clotting time (216.5 (104~300) sec vs. 223.5 (42~300) sec, p = NS), APACHE II scores (24 (7~34) vs. 24.5 (10~39), p = NS) and filter life span (15 h 57 min (5 h 5 min~71 h 47 min) vs. 17 h 18 min (3 h 40 min~71 h 32 min), p = NS) between two groups. Compared with baseline value of aPTT, its prolongation did not appear in 30 minutes after starting RRT in group A (from 37.9 (27.2~180) sec to 38.4 (25.3~180) sec, p = NS). However, aPTT significantly increased in group B (from 35.9 (27.2~97.2) sec to 38.9 (29.7~86.8) sec, p = 0.017) without clinical events. **Conclusions:** Priming with the higher dose of heparin and washing did not reveal the beneficial effect on filter life

but prolonged aPTT. It suggests that polyethyleneimine does not strongly enough bind heparin and requires heparin-coated filter where heparin can be adhered more tightly.

Tissue Engineering of Skin: Creating a New Bio-Artificial Organ for Clinical Application —Symposium

K13 (EI0433)

Tissue Engineering of Skin: Creating a New Bio-Artificial Organ for Clinical Application

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Large full-thickness skin defects resulting from burns, soft tissue trauma, congenital giant nevi, tumor resection, and disease leading to skin necrosis, represent a common and significant clinical problem that is far from being solved. The main challenges encountered are the following two**:** First, there is donor site shortage for autologous skin transplantation when the defect exceeds 50–60% of the total body surface area. The typical clinical example is a massive deep burn. Second, most conventional skin grafting techniques to provide autologous coverage are based on transplanting split-thickness skin (the today's gold standard). Split-thickness skin contains all of the epidermis but only part of the dermis, and that frequently leads to scarring. Rarely, scarring is mild and irrelevant. Often, particularly in children, there is hypertrophic scarring or keloid formation that is frequently disabling and disfiguring. There are still two major challenges concerning the development of novel skin substitutes: 1) On its way to an optimized and long lasting structure and function, a dermo-epidermal substitute has to be efficiently and appropriately vascularized. Attempts to reach this goal have entered a period of significant progress; however, a final breakthrough is still missing. 2) Much is still unknown about the mechanisms by which tissues form and heal, yet insights from developmental biology and other biological disciplines are already guiding the development of "instructive matrices" that work with nature's own mechanisms of organogenesis and repair. Biologically active matrices containing cells that constantly produce a physiological set of biologically active factors, in their appropriate concentrations and locations, in combination with secure, automated and highly reproducible techniques to produce a new generation of complex skin substitutes both, in a desired number and in a constant quality, are now the guidelines of modern "skingineering".

O74 (EI0416)

Single Epidermal-Dermal Scaffold for the Regeneration of Full-Thickness Skin Defects

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Objectives: Create a single tridimensional epidermal-dermal scaffold, using the spray-assisted Layer-by-Layer (LbL) to build a film on the top of a hyaluronan (HA) porous scaffold. **Methods:** The porous scaffold was created by mixing modified HA in the aldehyde and in the hydrazide forms, followed by freeze-drying. A film, which is intended to act as an epidermal membrane, was produced on the top of this scaffold by sequential deposition of HA and Poly-L-lysine (PLL), creating a polyelectrolyte multilayer. The film build up mechanism was studied by spraying the electrolytes on silicon wafer at pH 5, 6 and 7 up to 50, 100 and 150 bilayers (BL). The topography and roughness of the films was determined using atomic force microscopy (AFM), and its thickness by profilometry. The attachment and proliferation of human keratinocytes (hKc) on the films was observed under SEM and its metabolic activity measured over time. **Results and Discussion:** The films' thickness increased with the number of BL deposited, independently of the pH. The film created on the silicon wafer for 150 BL at pH 7 had a thickness of 1682.9 ± 291 nm and a roughness of 1.11 ± 0.38 nm. Also considering pH 7 as the most compatible for cell culture, this condition was selected to create the membrane on the top of the HA porous scaffold, in which hKc were able to attach and form a monolayer after 96 h of culture. **Conclusions:** A one step method to produce a single epidermal-dermal scaffold was established by spraying the $(PLL/HA)_{150}$ polyelectrolyte on the top a porous scaffold. The membrane, physically connected to the scaffold, allowed its colonization by hKc. This strategy confers cohesion to the epidermal-dermal substitute, and is expected to contribute to improved performance of the skin substitute by promoting the interaction between the cells present in both layers.

O75 (EI0365)

Boost of Epidermal Stem Cells from Adult Keratinocytes

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Objectives: Skin tissue engineering has the longest history of commercialization, however, skin analogues still lack in completely meeting the demands, especially in the cases of massive skin loss. The long-term function of the skin equivalents could be limited by the terminal differentiation of the grafted keratinocytes. Thus, the use of stem cells for this purpose, namely epidermal stem cells (EpSCs), would provide an active source of biological material. EpSCs isolation difficulty remains, mainly due to the lack of well-determined approaches and markers. This work integrates an assemblage of strategies to be pursued in order to accomplish enrichment of this multipotent fraction. **Methods:** Human primary keratinocytes (hKC) were isolated from human adult skin, and different methods for EpSCs fraction enrichment were applied. Rho-associated protein kinase (Rock) inhibitor Y- 27632 was firstly administered to freshly KC cultures to increase EpSC number. Consecutive selective methods, rapid adherence to b1-integrin ligand in collagen type IV and immunomagnetic separation methods, were then performed to establish populations based in the a6/CD71 expression. CFUs assay, flow cytometry and immunocytochemistry were then performed, focusing on the effect of the treatments over expression rate of early epidermal markers keratins19/5/14 and correlated with a6/CD71 subpopulations. **Results and Discussion:** Collagen IV treatment resulted in increased cell adhesion, and polygonal shape and small size cells. Rock Inhibitor, not only enhanced cell proliferation (k14+ cells), namely the keratinocyte stem cell fraction $a6^{+}/CD71^{-}$, but also raised the expression of K19. Additionally, a boost in the keratinocyte stem cell fraction $a6^{+}/CD71^{-}$ with the expected morphology and in higher cell number, particularly in the fraction that was subjected to the previous treatments, was observed after the subsequent CD71 and a6-integrin immunomagnetic selections. **Conclusions:** The methodology presented in this work indulges the boosting of EpSCs in hKC culture, with a consecutive purification and separation from hKC bulk.

O76 (EI0308)

Development of a Vascularized Skin Equivalent

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Objectives: Due to the lack of an analogue for the vascular system, current skin equivalents (SE) cannot be used to test the capacity of a given substance to penetrate through the skin into the bloodstream. However the integration of a vascular system into a SE would amplify the possible applications in research fields such as toxicity testing or oncology, by providing a model for the critical barrier between the skin and the vascular system. The objective of this study is to integrate a full thickness SE into a biological vascularised scaffold (BioVaSc), based on an acellularized part of a porcine jejunum. This BioVaSc can already be used for the formation of renal and liver tissue and was successfully implanted into a patient as a trachea patch. **Methods:** Primary human keratinozytes and human fibroblasts were seeded on the BioVaSc and cultured under submersed conditions for seven days. To initiate the differentiation of the keratinocytes, the construct was subsequently cultured at an air-liquid interface for another 12 days. The formation of skin tissue on the vascularized scaffold was determined using hemalaun/ eosin (HE) and immunohistological staining. **Results:** Histological HE and immunohistological staining revealed a stratified epidermal layer of keratinozytes with a corneous layer on one top of the BioVaSc and equally distributed fibroblasts inside of the scaffold. Thus we could show that the BioVaSc provides a suitable microenvironment that facilitates the formation of a functional SE. **Conclusions:** In this work we demonstrated that the BioVaSc is a suitable scaffold for a SE. In future experiments we will combine the vascularized skin substitute with a new developed bioreactor that enables the supply of the vascularized skin substitute through the vascular system and the culture at an air-liquid interface.

O77 (EI0080)

Engineering a Functional Microvasculature Within a Dermo-Epidermal Skin Substitute

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Objectives: The development of rapidly and efficiently vascularized tissue grafts is vital for tissue engineering. Especially within the first days after transplantation, rapid establishment of an intact vascular network and blood flow often decides whether or not a graft is taken. One way to accelerate vascularization of an enginered tissue is to provide it with a pre-formed vascular

network. The fundamental idea behind this approach is that fast anastomosis of a preformed vascular network with the patient's vascular system can compensate for the delayed neovascularization, which usually results in a shortage of blood supply right after transplantation. Here we describe the generation of an engineered skin substitute, from a single human skin biopsy, displaying a network of functional and anastomosing capillaries. **Methods:** Primary human endothelial cells, fibroblasts and keratinocytes were isolated from human skin biopsies and expanded in vitro. These cells were used to generate a prevascularized dermo-epidermal skin substitute based on fibrin hydrogels. To test the effect of prevascularization, the skin substitutes were transplanted on the back of immuno-incompetent rats. The quality of the engineered skin was evaluated by excising the grafts after different time-points and subsequent analysis. **Results:** The microvasculature produced within an organotypic skin substitute consists of a high number of branching and continuously lumenized capillaries. After transplantation, anastomosis with the rat vasculature occured and the differentiation process of the in vitro generated microvascular structures continued by the attraction of mural cells, which are known to support stabilization and maturation of capillaries. **Conclusions:** We show that a network of branching and continuously lumenized capillaries within a dermo-epidermal skin substitute can be produced in vitro, and that prevascularization of tissue substitutes (derived from fibrin hydrogels) is demanding but possible.

Cardiovascular General 5: Device & Biology—General Session

O78 (EI0372)

First Step towards the Generation of a Vascularised 3D—Cardiac Construct

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Objectives: *In vitro* generation of bio-artificial cardiac construct (BCC) represents a promising method for the repair of ischemic heart tissue. Deficiency of oxygen and nutrient supply in the centre of 3-dimensional tissues may be addressed by *in vitro* vascularisation. Here we investigate whether tubular-like network built by endothelial cells in a solid BCC, could be supplemented by externally applied endothelial cells through the BioVaM (Biological Vascularised Matrix), to generate a potential perfusion system. **Methods:** BioVaM were decellularized using a protocol established in our lab. GFP-labelled rat heart endothelial (RHE) cell line were infused into the venous and RFP-labeled RHE cells were infused into the arterial vessel bed of the BioVaM. BCC were generated with a mix of isolated rat neonatal heart cells, collagen I, and Matrigel and casted onto the BioVaM, which was already preseeded with GFP / RFP labelled RHE cells. These constructs were cultivated for 10 days, and thereafter prepared for morphological analyses, performed via Confocal Laser-Scan-Microscopy (CLSM). Crysections were analyzed using fluorescent microscopy. **Results:** Using a CLSM both cell types, GFP / RFP labelled RHE cells, were determined within the 10 days cultivated BCC-BioVaM complex. RHE cells were repopulating preexisting vessels of the BioVaM, but most of these cells could be found through the whole thickness of the BCC. A dense, highly organized tubular-like branching within the BCC resembled to a capillary network, where red-labelled RHE cells derived from arteries and green-labelled RHE cells from veins. Both cell types also connected to the $CD31^+$ endothelial cells being a cellular component of BCC. **Conclusions:** Invasion and capillary-like formation of externally added GFP / RFP labelled RHE cells from the BioVaM vessels into the cardiac construct represent an important step towards the engineering of a functional perfusion system, and thus vascularised and well-organized thick cardiac construct.

O79 (EI0379)

Coronary artery Calcifications and Cardiovascular Mortality in Hemodialysis Patients

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Objectives: Coronary Artery calcifications are frequent in renal patients and are related to significant cardiovascular morbidity and mortality. The possible relationship between coronary artery calcifications and overall cardiovascular mortality was investigated in maintenance hemodialysis (mHD) patients **Methods:** Two hundred five (105 males and 100 females aged 59.85 ± 12.77years, on mHD since 62.30 ± 55.00 months) were enrolled into the study. All patients underwent a single cardiac multilayer spiral computed tomography (MSCT). Calcium load was quantified according to the Agatston score (AS; Agatston AS, JACC 1990). According to AS patients were then stratified into groups 1 (AS = 0), 2 (AS 1 to 400), 3 (AS 401–1000) and 4 (AS > 1000). All patients were followed between January 2003 and January 2011. Primary endpoint of the study was mortality for a major acute cardiac event. Seven-year actuarial survival was calculated for patients of the four groups separately by Kaplan-Meyer equation. Patients who died for causes other than cardiovascular disease and transplanted patients were censored. The log rank test was employed to compare survival curves. **Results:** One hundred two patients (49.7%) died for cardiovascular disease during the follow up Seven-year actuarial survival was more than 90% for patients of groups 1 and 2, but failed to about 50% in patients of group 3 and to <10% in patients of group 4. Hence, patients with AS > 400 show a significantly higher cardiovascular mortality compared to patients of with AS < 400 ($p < 0.0001$). **Conclusions:** The pathogenesis of arterial wall calcifications involves apoptosis and an osteoblastic-like transformation of smooth muscle cells that induces the synthesis of bone matrix and a local chemotactic activation of mineralization processes within the vascular wall. The presence of extended coronary artery calcifications detectable with cardiac MSCT may be predictable of an elevated risk of cardiovascular mortality at least in mHD patients.

O80 (EI0294)

Novel Pseudo-Aneurysm Model—Using a Biodegradable Polymer

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Objectives: Several experimental aneurysm models exist which are mainly used to test new interventional methods for obliteration and/or exclusion of the aneurysms. However, creation of a pseudo-aneurysm formation has not been described. We present a novel method to induce pseudo-aneurysm by implanting a biodegradable vascular prosthesis in the infrarenal rat aorta. **Methods:** In 6 anesthetised rats a polydioxanone (PDO 2 mm-ID; 15 mm-length) vascular prosthesis was implanted end-to-end in the infrarenal aorta and compared to 6 control rats with ePTFE prostheses. After 3 weeks a pan-angiography followed by graft explantation for histologic assessment was performed. The widest diameter of the graft was compared to the size of the native aorta. **Results:** One animal died of ruptured abdominal aneurysm on day 20, one animal did not develop an aneurysm and the four remaining showed an increase in diameter of 256%. Autopsy showed saccular pseudo aneurysms. SEM and histology revealed fragmentation of the prosthetic material due to degradation, covered by a fibrous capsule containing parietal thrombus. All ePTFE controls were patent and showed no aneurysmal dilatation, nor pseudo-aneurysms. **Conclusions:** Fast degrading synthetic polymers such as PDO will dilate, rupture and form pseudo-aneurysms after a period of 3 weeks, if implanted in the abdominal rat aorta with systemic pressures. This new aneurysm model may be of interest for testing new interventional treatment modalities.

O81 (EI0142)

Acoustic Detection of Initial Thrombosis Formation in a Novel Heart Valve Test Rig

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Objectives: Even with the use of anticoagulants, thrombosis is still one of the major problems of mechanical heart valves. Investigating flow induced thrombus formation initial aggregates have to be determined. Laboratory parameters and pressure curves were not able to detect initial thrombus formation in this test setup, therefore acoustic methods were implemented. **Methods:** Minimally heparinized porcine blood was used in a novel test rig that mimics the left ventricular anatomy and hydrodynamic conditions for thrombosis enquiries with the Saint Jude Medical bileaflet valve. Opening and closing sounds were acquired using a hydrophone (Type 8103; Bruel&Kjaer) placed proximal to the aortic root. Sound signals were processed using PULSE (Type 3560B; Bruel&Kjaer) and MATLAB. First, parameters in time and frequency domain were generated and evaluated using artificial silicon thromboses placed at positions of concern. Parameters independent from working conditions were selected. A Naive Bayes Classifier and an Artificial Neural Networks with three layers and 20 neurons were applied for thrombosis detection via pattern recognition. Six subsequent blood tests provided optimization of the classifiers. **Results:** Naive Bayes Classifier showed a sensitivity of 85% for thrombosis detection at the initial blood test. Classifier optimization over 6 blood tests increased sensitivity to 95%. Artificial Neural network could not be optimized by the blood tests and achieved a sensitivity of 76%. **Conclusions:** Detection of initial thrombosis formation in vitro via sound analysis has proven a successful tool. Naive Bayes Classifier was found to be the more sensitive classifier and can be implemented to other valves. Future work will focus on the development of methods to detect the location of thrombus.

O82 (EI0181)

Age Releated Changes in Biomechanical and Morphological Properties of Transgenic Porcine Valve Dedicated for the Use in Tissue Engineering Applications

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Objectives: Because of the imbalance of the donor and recipient the cardiac valves derived from pigs do not express the galactose 1, 3 galactose (-Gal) antigen, are attractive source for the preparations of tissue engineered heart valve. Due to the differentiation of recipient there is a need for the preparations of different size valve prosthesis. The aim of the study was to estimate how the age and weight of the transgenic porcine can influence the morphological and biomechanical properties of the valve prosthesis, those how the size of the bioprosthesis, dedicated for different recipient can be controlled. **Methods:** The morphological and biomechanical properties of acellular aortic and pulmonary valve in relations to the age and weight of transgenic porcine was tested. Morphology of the valve was analyzed using H&E and Masson staining. Uniaxial tensile test was used to estimate the biomechanical properties of the examinated valve. The computer simulations based on Finite Elements Methods (FEM) was used to study the influence of the decellularizations procedure on the hemodynamic conditions. **Results:** The differences in the morphology and biomechanical properties of the acellular pulmonary and aortic valve were observed. Uniaxial tensile test demonstrated that the energy to break, peak load or peak stress of the aortic valve increase in relations to the weight and age. For the pulmonary valve the value of this parameters ware constant, they were independent to weight and age of the animals. The value of biomechanical parameters was significantly lower for the pulmonary valve compared with the aortic valve. **Conclusions:** The aortic valve derived from transgenic porcine are more valuable from the preparations of different size bioprosthesis**.** The use of tissue engineered pulmonary valve as an aortic valve replacement can be strongly limited.

O83 (EI0006)

In-Body Blue Light Illumination Realized the Formation of Functional and Robust "Biotube" Vascular Grafts with Many Capillaries and Elastic Fibers

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Objectives: The autologous "biotube," developed by using in-body tissue architecture technology, is one of the most promising small-diameter vascular grafts in regenerative medicine. The walls of the biotubes obtained by a traditional silicone mold-based method were very thin, and this is still the primary obstacle while handling anastomosis, even though these biotubes have adequate mechanical properties. The aim of this study is to evaluate the effect of optical stimulation of subcutaneous tissue formation in the body during the preparation of the biotubes. **Methods:** A blue light-emitting diode (LED) and a small battery were embedded into a silicone rod as a luminescent mold (diameter 5 mm; length 50 mm). The biotubes were prepared by placing the molds into the dorsal subcutaneous pouches of beagle dogs (each weighing about 10 kg) for 2 weeks with initial 2-days illumination. **Results:** The wall thickness of the obtained biotubes was 506.9 ± 185.7 μm, which was about 7 times thicker than that of the previous biotubes prepared by 2 months of placing silicone molds without illumination. Smooth muscle specific α-actin positive cells migrated in the wall and many mature capillaries with smooth muscle cells were markedly observed in the middle layer of the wall. Very interestingly, the formation of elastic fibers was firstly observed only at two weeks along with collagen fibers mostly with a regular circumferential orientation. The resulting optical-stimulated biotubes could be auto-implanted in the carotid arteries without any stress in handling of anastomosis as well as native one. **Conclusions:** The short-term in-body optical stimulation resulted in the formation of robust biotubes with vascular components of smooth muscle cells and elastic fibers in addition to collagen fibers.

Citrate Anticoagulation—A Future Option for Extracorporeal Blood Purification—Symposium

O84 (EI0436)

The Use of Citrate Containing Dialysate for Anticoagulation in Hemodialysis (HD). Report of Clinical Experience

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The blood membrane interaction induced during haemodialysis (HD) activates several cascade systems including the coagulation system, inducing clotting. To prevent from such clotting and to keep patency of the dialyzer, anticoagulation using heparin or low molecular weight heparin (LMWH) such as tinzaparin is used. As an adverse effect, the use of these anticoagulants, will increase the risk of bleeding. To avoid this various settings are available such as administration of citrate either intravenously or by local administration at the site of the dialyzer. The latter technique has been used for some years in the USA, while there is less experience in Europe. In this clinical setting 15 patients were randomized to a crossover using either LMWH-tinzaparin and a series of halved dose of tinzparin and local citrate anticoagulation using Citrasate (Cit), provided by Scandinavian Medical (Kista, Sweden). The study included analyses of subjective patency, ionized calcium (iCa), Kt/V. During Cit-HD the iCa was significantly more reduced with prolonged time. The lowest iCa measured was 0.96 mmol/l. The median iCa after 210 min of HD was for Cit-Hd 1.02 and for tinz-HD 1.16 ($p = 0.001$). Patency of dialyzers was estimated as clear in 9/15, stripes of clotted fibers in 5 and a red filter in 1 HD. In a second series, after further reduction of tinzaparin, patency was clear in 2/11, stripes in 5 and red in 4 of which 3 HD had to be prematurely interrupted. In conclusion the data indicate that local citrate anticoagulation may help to reduce or eliminate the use of heparin or LMWH for dialysis. This may help in preventing the risk for bleeding, especially in the course of surgery. However, optimization of doses of anticoagulants together with Citrasate have to be individualized.

O85 (EI0432)

Why Citrate Anticoagulation Will be the Future Anticoagulation in Chronic Haemodialysis

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Objectives: Cardiovascular diseases have a very high impact in mortality and morbidity of patients suffering from end stage liver disease being under chronic haemodialysis therapy. It is well known and clinically proved that chronic and acute inflammations are processes which are responsible for cardiovascular diseases, especially related to arteriosclerosis. Especially in RDT patients treated three times a week by haemodialysis—inflammatory processes are more or less continuously activated by the use of extracorporeal circulation using materials which are responsible for activation of inflammatory processes. **Methods:** Citrate anticoagulation is a possibility to diminish or even completely inhibit those inflammatory processes during haemodialysis in case of using the optimal concentration of ionised calcium in the extracorporeal circuit. Using this optimal target concentration there is a possibility to inhibit the activation of the alternative pathway of the complement system. Furthermore, by complete inhibition of the activation of the coagulation system any link to other inflammatory systems like complement systems or even kinin systems are blocked. Additionally, there is also the possibility to block the release of microparticles from endothelial cells or even blood cells like platelets or white blood cells, which are also involved in inflammatory processes being responsible for genesis of arteriosclerosis. **Results:** By analysis of concerning literature it will be shown that by blocking any kind of inflammatory processes during haemodialysis there is a real chance to diminish cardiovascular complications in RDT patients and, therefore, to decrease the mortality but also morbidity of this patients. **Conclusions:** Therefore, citrate anticoagulation should also be considered as a future anticoagulation method in ESRD-patients treated with haemodialysis regularly.

O86 (EI0431)

Targeting Ionized Calcium—a Essential Tool in Citrate Anticoagulation

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Objectives: Citrate anticoagulation (CA) is an upcoming method of anticoagulation (AC) in extracorporeal blood purification (ECBP). It offers several avantages such as prolonged filter life time, suppression of the complement activation, circumvention of HIT (heparin-induced thrombocytopenia) and the limitation oft he anticoagulation to the extracorporeal circuit (regional AC). However, to achieve these advantages, the ionized calcium (iCa) concentration has to be kept within a certain range and accurate monitoring is mandatory. Aim of this study was to show that target-oriented CA offers a flexible and safe AC for patients in ECBP. **Methods:** In vitro as well as in vivo studies were carried out with different target concentrations for iCa. Activated clotting time (ACT) and complement activation (C3a) were measured and correlated with the iCa. **Results:** Our results show an excellent correlation between ACT and iCa. The complement activaion can effectively be suppsessed at iCa concentrations of ≤0.2 mmol/l. **Conclusion:** Target oriented CA offers a very high flexibility in ECBP and enables an individually adapted AC based on the patients' needs.

O87 (EI0347)

Citrate Anticoagulation for Different Hemodialysis Procedures

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Citrate has many characteristics of the ideal anticoagulant for hemodialysis. The major issue preventing its wider use is the complexity of current citrate anticoagulation protocols. From 1993 we use 4% trisodium citrate for hemodialysis, plasma exchange and continuous renal replacement therapy, prepared by our hospital pharmacy. Calcium-free dialysate is used, with sodium and bicarbonate concentration usually reduced on hemodialysis monitor. In the last years we perform more than 3000 citrate procedures per year, approximately 9–10 citrate procedures daily. The majority, approximately 60% of citrate procedures are performed in chronic hemodialysis patients, the rest of them acute patients, mainly in intensive care units. In chronic hemodialysis patients, citrate anticoagulation is usually performed for a limited time, when increased bleeding risk exists. However, in our center we have treated 16 patients with long-term (>3 months) citrate anticoagulation, with a maximum duration of 6.5 years, without significant side effects. Besides standard bicarbonate hemodialysis we have successfully used citrate for single-needle hemodialysis, predilutional on-line hemofiltration and plasma exchange. Special protocols were designed for each procedure. The use of calcium-containing dialysate was associated with significant clotting in venous bubble trap, both during hemodialysis as well as hemodiafiltration, despite higher citrate dose. Predilutional on-line hemofiltration was the only procedure with calcium-containing infusate (1.25 mmol/l) and successful regional citrate anticoagulation. In the last year we have successfully used citrate for prolonged, 8-hour high cut-off (Theralite, Gambro) hemodiafiltration in patients with plasmocytoma or rhabdomyolysis. If citrate accumulation and/or alkalosis occur, we perform short heparin-free dialysis to remove citrate and correct alkalosis. Despite its complexity, citrate anticoagulation is safe if performed by trained nurses and precise protocols. It offers many advantages over other anticoagulation methods. We can expect increase in the use of citrate in future both in intensive care units as well as in chronic hemodialysis patients.

Latest Advances in Preventive and Regenerative Medicine Technologies—Symposium

K14 (EI0413)

Instructive Membranes for Neuronal Regeneration

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Many strategies are aimed to develop biomaterials for supporting and inducing neuronal regeneration. Polymeric semipermeable membranes are attractive for their high selective properties for creating the microenvironment in order to promote neuron adhesion and growth. Micro- and nanostructured membranes would be able to modulate the adhesion, proliferation and differentiation of cells, which are fundamental processes for tissue regeneration by governing the mass transfer of molecules that generate a precisely controlled microenvironment mimicking the specific features of in vivo environment. Membranes may guide the axon regeneration with the surface geometry by controlling the mass transfer of molecules between the cell microenvironment and the external milieu providing to the cells chemical, physical and topographical features similar to those of the complex in vivo extracellular matrix through patterns of chemistry and topography from macroscale to nanoscale. In addition, the membrane surface can be tailored with proteins, peptides and cell-specific recognition factors by modification processes in order to stimulate specific cell responses and maintain differentiated functions. In this paper the author will present the controlled design and preparation of polymeric membranes with appropriate physical, chemical and biological cues, which are relevant to induce the neuronal regeneration. In particular the influence of membrane configuration (e.g., flat, tubular), surface properties (e.g., roughness, pore size, porosity, topographical features), and physico-chemical properties (e.g., wettability) on neuronal outgrowth and differentiation as well as the membrane ability to reconstruct the neuronal network will be discussed. Neurite outgrowth and the orientation of cellular growth, which are two important processes, can be facilitated by designing a well-defined cellular pattern. Recent results in the development of synthetic and biodegradable membranes with tailored physical, chemical and morphological properties, which are engineered to stimulate neurite outgrowth, will be discussed.

K15 (EI0443)

Self-Organzation in a Culture Dish

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Objectives: To develop a model of cellular self-organization in the laboratory Methods: Mouse embryonic fibroblasts (MEFs) cultured in 3-dimensional soft scaffolds. Real time PCR, western blot, immunohistochemistry. **Results:** Cellular self-organization studies have been mainly focused on models such as Volvox, the slime mold *Dictyostelium discoideum*, and animal (metazoan) embryos. Moreover, animal tissues undergoing regeneration exhibit properties such as cellular dedifferentiation and self-organization processes that ends in rebuilding tissue complexity and function. We speculate that the recreation *in vitro* of the biological, biophysical and biomechanical conditions similar to those of regenerative milieu could elicit the intrinsic capacity of differentiated cells to proceed to the development of regenerative structure. In this presentation I will show that when MEFs are cultured in a soft nanofiber scaffold they establish a cellular network that causes an organized cell contraction, proliferation and migration that ends in the formation of a tissue structure that recapitulates certain aspects of early development, such as temporal control of early embryonic genes followed by tissue determination. Interestingly, a subset of early mesodermal genes (*Brachyury*, *Sox9* and *Runx2*) were up-regulated during this morphogenetic process. Interestingly, the expression of *Brachyury* determined the formation of an early mesoderm-like tissue followed in time by expression of Sox9 and Runx2, which resulted in the spontaneous formation of cartilage-like tissue. **Conclusions:** Since cellular self-organization is an intrinsic property of tissues undergoing development this new experimental paradigm could bring new ways to obtain functional tissues in a dish.

Intra-Aortic Balloon Pump as a Cardiac Assist Device—Symposium

K16 (EI0345)

Shape Change of the Intra-Aortic Balloon: Can it Offset the Operational Deficit at Angles to the Horizontal?

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Methods: Three balloons; cylindrical, 40 cc, tapered increasing diameter balloon, 37 cc (TiD) and tapered decreasing diameter (36 cc TdD), have been compared in a mock loop at 0°, 20° and 30°. Pressure at 7 positions along the balloon, and flow rate on either side of the balloon during inflation and deflation were sampled simultaneously at 2 kHz. The ratios (R_{inf} and R_{def}) of water volume displaced towards and away from the tip of the balloon to the total volume displaced during inflation and deflation respectively, and time of maximum pressure (TMP) at each position were determined. **Results:** R_{inf} for TdD and 40 cc is decreased (51.28% vs. 44.96%) and (49.34% vs. 44.21%) respectively, while it is increased for TiD (49.26% vs. 50.14%) when angle changed from 0° to 30°. R_{def} decreased by 13.77% for TdD, 11.57% for 40 cc and 5.12% for TiD when angle changed from 0° to 30° respectively. TMP of TiD and 40 cc balloons remained approximately unchanged with increasing angle; occurring earlier at the base than the tip of the balloon (TiD) and vice versa (40 cc). TMP of TdD occurred almost simultaneously along the balloon at the horizontal position, increasing to a time-lag of 5 ms and 12 ms between tip and base with increasing angle to 20° and 30° respectively. **Conclusions:** R_{inf} and R_{def} of TiD are less affected by angulation than those of TdD and 40 cc. TMP of TiD is also less affected by angulation compared to the 40 cc and TdD. The shape of TiD appears to provide overall better inflation (R_{inf}) and deflation (R_{def}) benefits in vitro, which requires dimensional changes and in vivo investigation to establish the enhanced benefit of the newly shaped IAB.

O88 (EI0121)

Effects of Baroreflex Activities on Iabp Hemodynamics in a Closed-Loop Hybrid Cardiovascular Model

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Objectives: aim of this work is the integration of the autonomic mechanism of pressure regulation during temporary IABP assistance in a hybrid circulatory model. **Methods:** The hybrid model is based on merging computational and hydraulic models. The lumped parameter computational model includes the upper thoracic aorta, circulatory districts (upper body, kidneys, splanchnic, lower body, pulmonary, coronary circulation) and left/right hearts. The hydraulic model provides a representation of the lower thoracic aorta by a silicon rubber tube containing a 40 cc IAB. An additional numerical module provides a representation of the baroreflex mechanism in terms of afferent and efferent

sympathetic nerve activity (ANA, ENA). Baroreflex model acts as a feedback control loop that regulates the blood pressure by changing heart rate (HR), peripheral resistance and venous tone of each circulatory district. Experiments were conducted applying IABP assistance to a pathological circulatory condition. **Results:** The increment of diastolic pressure due to IABP provides an increment of ANA (+7%) and a decrement of ENA (–9%). Operating the IABP induced a reduction in HR by –6% (90 vs. 95 bpm), in kidney and upper body resistances by –5% (5.43 vs. 5.72 and 5.17 vs. 5.44 mm Hg·s/ml, respectively). IABP also induced an increment in kidney flow by +7% (0.63 vs. 0.59 l/min) and upper body flow by +6.8% (0.50 vs. 0.46 l/min). By switching the IABP assistance frequency from 1:1 to 1:2 or 1:3 the mentioned effects reduce progressively. Results indicate that the short term effects of IAB are small, even in the presence of a model including baroreflex control. **Conclusions:** the model provides an instrument for the assessment of IABP effects on baroreflex mechanism due to the increase of mean diastolic blood pressure. This contributes to predict and study the global evolution of hemodynamic condition after IABP activation and the resultant change in organ flows.

O89 (EI0187)

Intra-Aortic Balloon Pump: Indications for Use

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Objectives: The IABP has a long record in supporting patients after myocardial infarction or cardiac surgery. So far, the effects of IABP therapy have been investigated, but mainly in small observational studies and animal experiments, with conflicting results and variable magnitude of the results. Lack of well defined indications for use might explain these findings. To enhance its clinical efficiency and to better define indications for use, advanced models are required for testing the interaction of the IABP with patient specific factors of the cardiovascular system. **Methods:** A patient having mild blood pressure depression (90/50 mm Hg) and a lowered cardiac output was modeled in a lumped parameter model and a model-controlled mock circulation, both featuring a complete systemic, pulmonary and coronary vascular bed. The IABP was numerically modeled as a cylinder-shaped collapsible tube, while a real IABP was used in the mock circulation. IABP support was applied with the standard in clinical practice used timing settings, while the support capabilities of the IABP in terms of cardiac output, coronary flow, cardiac stroke work and mean aortic pressure were evaluated for different levels of ventricular contractility, heart rate and aortic compliance. **Results:** Ventricular contractility, heart rate and aortic compliance appeared to be major determinants of IABP performance. IABP support showed more pronounced advantages in a clinical scenario of deep cardiogenic shock, than in a scenario of only mild blood pressure depression and a slightly lowered cardiac output. Increase in heart rate ultimately interfered with the time required for complete in- and deflation of the balloon, while a very distensible aortic wall permitted free wall motion in response to the augmented blood pressure. **Conclusions:** The use of IABP therapy might be reserved for patients in deep cardiogenic shock, having a stiff aorta and a heart rate not exceeding 140 min^{-}.

O90 (EI0057)

Balloon-Associated Impact on the Perfusion of Visceral Arteries after the Insertion of an Intra-Aortic Balloon Pump (IABP)

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Objectives: The IABP is worldwide the most commonly implanted extracardiac, mechanical device in the treatment of patients with acute coronary syndrome and during cardiogenic shock. A CT scan confirmed malposition of the balloon—in this case an occlusion of visceral arteries—led us to start a systematic analysis of cardiac surgical patients. We analyzed the frequency of the malposition, its cause and the resulting clinical relevance. **Methods:** From January 2007 to March 2009, a total of 621 of 7756 cardiac surgical patients (8.0%) received perioperative IABP support, of whom 63 (10.1%) received a thoracoabdominal CT during IABP support. Proximal and distal balloon positions were analyzed. The anatomic distance from the left subclavian artery to celiac trunk and aortic transverse diameter were measured and compared with implanted balloon dimensions. Mean age was 67.1 ± 11.9 years; 33.3% were female, and height was 169 ± 9 cm. **Results:** Based on radiography, proximal balloon position was correct in 96.8% but only appropriate in 38.1% based on CT. In 61 of 63 patients, compromise of at least 1 visceral artery was found: celiac trunk, 96.8%; superior mesenteric artery, 87.3% and renal arteries, 66.7%. Left subclavian artery to celiac trunk distance was 241 ± 23 mm, and balloon length was 248 ± 17 mm and corresponded to an anatomic to balloon length mismatch in 68.2%. Spinal deformations were found in 42.9%. Laparotomy for mesenteric ischemia was required in 23.8%. Hospital mortality rate was 60.3%. **Conclusions:** In all of our patients the IABP was implanted according to the current implant procedure guidelines. In 96.8% of the patients, however, we found via CT scan a malposition of the proximal balloon end as well as an anatomic mismatch between aorta and the length of the balloon. For clinical reasons it is highly recommended to implant a shorter balloon length than the one which has been recommended so far.

O91 (EI0055)

How Should I Wean Intra-Aortic Balloon Pump? Differences in Hemodynamic Response between Progressive "Volume-" and "Rate-" Weaning.

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Objectives: To evaluate the best method to wean a patient from intra-aortic balloon pump (IABP) after cardiogenic shock **Methods:** Thirty consecutive patients needing intraoperative IABP because of cardiogenic shock were enrolled in the study and randomized in the Intensive Care Unit (ICU) to 2 different weaning protocols. Fifteen patients were randomized to be weaned by ratio (4 consecutive hours of 1:2 assisting ratio followed by 1 hour of 1:3 ratio; Group-R), 15 by progressive volume deflation (10 cc every hour for 5 consecutive hours; Group-V). Weaning protocol started if cardiac index >2.5 l/min/m^2, CVP ≤ 12 mm/Hg, blood lactate <2.5 mmol/L, mean arterial pressure >65 mm/Hg with preserved diuresis lasted at least 5 consecutive hours. Five-hours were "a priori" set as weaning duration. IABP lasting >5 hours defined "failure". Pressure recording analytical method (PRAM) collected cardiac index (CI), indexed systemic vascular resistances (SVRI), and cardiac cycle efficiency (CCE) at 8 different time points (T1 to T5 for the first 5 weaning hours, T6: 2 hours postwithdrawal; T7: 12 hours post-withdrawal; T8: ICU-discharge). Central venous pressure (CVP) at same time-points, time from IABP-withdrawal to ICU-discharge and weaning failure were also recorded. Perioperative troponin-I and lactate leakage were compared. Repeated-measures ANOVA assessed group, time and group*time interactions. **Results:** All patients were successfully weaned and discharged home. Group-V showed better preserved CI, CCE and CVP (group*time p = .0001 for all). Group-R had worse CCE since T3 to T8 (p ≤ .001), CVP since T4 to T8 (p ≤ .0001) and CI since T5 to T8 (p ≤ .0001). SVRI proved comparable during the entire weaning period (p = NS). Despite no differences were detected in troponin-I leakage, lactate proved lower in V-group since T5 to T8 (p ≤ .027). Time from IABP-withdrawal to ICU-discharge proved longer in Group-R (p = .0001). **Conclusions:** Despite the quite similar clinical outcome, weaning the IABP by volume deflation after cardiogenic shock better preserved the haemodynamic parameters.

Artificial Organ Transplantation—Symposium

K17 (EI0030)

Limitations in Cardiovascular Ort

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Objectives: Cardiac and/or pulmonary transplantation is limited due to organ shortage, despite being the best long-term option. Therefore, major efforts are currently carried out to improve artificial or bio-artificial organs in the field of cardiovascular surgery. However, clinical results of small caliber, synthetic vascular prostheses are still sub-optimal. Besides the engineering and power miniaturisation, the main hurdles to overcome remain bio-compatibility, liability of infection and thrombogenicity. Our aim therefore is to develop a novel, synthetic, biodegradable tissue engineered vascular prosthesis. **Methods:** Cardiovascular patches and prosthesis have been developed by random nano-fibre electro-spun polycaprolactone (PCL) with and without drug additions and compared to ePTFE prostheses. *In vitro* tests included tensile, suture retention and cell growth. *In vivo* tests included thrombogenicity assays in the arteriovenous shunt (AVS) pig model, followed by vascular replacement in the rat abdominal aorta and in the pig carotid artery. Assessment included patency, compliance, thrombogenicity, cell ingrowth (endothelialisation and matrix formation) as well as degradation. **Results:** The *in vitro* mechanical properties and cell compatibility tests were better than ePTFE prostheses. *In vivo* thrombogenicity in the AVS showed an uptake of indium-labelled thrombocytes due to the porous structure. Long-term implants in the rat showed excellent patency up to 18-months with endothelialisation, cellular ingrowth and matrix formation. However, calcification and regression of cellularity and angiogenesis can be seen beyond one year of implantation and in the pig model micro- and macro-thrombus formation is seen at one month follow-up. **Conclusions:** Despite the very promising manufacturing, mechanical *in vitro* testing and *in vivo* results up to one year, the biodegradable electrospun PCL prostheses showed some late limitations, similar to the clinically-used ePTFE vascular prostheses, such as calcification and thrombus formation in cardiovascular applications.

O92 (EI0387)

The βair: Implantable Bio-Artificial Pancreas (Bap)

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Objectives: Current therapy for insulin-dependent patients requires frequent blood glucose testing and insulin injections. Quite often, this treatment does not result in optimized control over blood glucose. Islet transplantation could form the optimal solution for these patients once two key targets are met: adequate oxygenation and efficient immune protection of the donor tissue. The sub-dermal implanted βAir is designed to overcome both hurdles. **Methods:** The βAir is a two compartment device. Donor islets of Langerhans immobilized in a flat sheet hydrogel and an oxygen chamber separated from the islet module by a gas permeable membrane. The immune barrier is a three-layer system: a 0.4 µm hydrophilyzed Teflon membrane, that prevents cell-cell contact, thus preventing cellular immunity; a small pore size alginate hydrogel impregnated into the Teflon membrane, significantly inhibiting inward diffusion of immune macromolecules and a large flat alginate hydrogel inflicting with the capacity of cytokines and NO to affect the islets. **Results:** The βAir was implanted in small and large animals. Average blood glucose levels were adjusted to near normal for up to 6 months in iso and allogenic diabetic rats and for one month in large xenogenic pig. Upon retrieval, blood glucose levels returned to the disease state. No porcine DNA was found within the device. Migration of IgG across the impregnated membrane was reduced by 20 fold, suggesting a strict delay in penetration of antibodies and other components of the immune system. More than 90% of the alginate was maintained with minor loss of the cross linking ion, suggesting a stable alginate gel. **Conclusions:** Results demonstrate the ability of the βAir to treat diabetes in rats and pigs with long-term immuno protection.

O93 (EI0324)

Microwell Scaffolds for Extrahepatic Islet of Langerhans Transplantation in Type 1 Diabetes

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Objectives: The conventional therapy for type 1 diabetes is insulin administration. Despite this, some patients are poorly controlled and suffer from hypoglycemia and long-term complications. For these patients, allogeneic islet transplantation into the liver has become an alternative therapy[1]. Patients benefit from this therapy due to near normalization of blood glucose levels without an increased risk of hypoglycemia. However, islet graft function in the liver tends to decline over years indicating that the liver is not an optimal transplantation site[2]. In order to develop alternative transplantation sites with better long-term outcome, we have developed a new microwell scaffold platform. **Methods:** Microwell scaffolds were prepared from dense solution-cast and porous electrospun and salt-leached 4000PEOT30PBT70 block-copolymer films using microthermoforming. Polymer wettability and topology were assessed by captive bubble contact angle measurements, atomic force microscopy (AFM) and scanning electron microscopy (SEM). Furthermore, constructs were characterized for their permeability for the nutrient glucose. To determine the applicability of the constructs for islet transplantation, the morphology and function of human islets (three different donors) were studied after 7 days of culturing in the construct using SEM, histological analysis and glucose challenge tests. **Results:** We fabricated reproducible dense and porous films. The polymer films were hydrophilic (contact angle 39° ± 2°). Diffusion tests revealed that electrospun and salt-leached scaffolds were permeable for glucose. Based on SEM and histological analysis there were no indications for islet spreading or outgrowth of islet stromal cells. Function tests revealed that human islets remained responsive to glucose challenge after 7 days of culturing in the constructs. **Conclusions:** This study reports on the development of a novel microwell scaffold platform for extrahepatic islet of Langerhans transplantation. Alternative transplantation sites using biomaterial scaffolds may improve islet transplantation outcome.

O94 (EI0319)

Do the Parameters of Encapsulation of Langerhans Islets Influence on Insulin Secretion?

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Objectives: Diabetes remains a devastating disease, with tremendous cost in terms of human suffering and healthcare expenditures. A bioartificial pancreas has the potential as a promising approach to preventing or reversing complications associated with this disease. The immunoisolation of Langerhans islets have been developed as a method of normalization of the carbohydrate metabolism for diabetes. In this study we investigated the production of small diameter alginate microbeads (i.e. <0,3 mm) using electrostatic droplet generation. The aim of our study was to evaluate whether parameters of electrostatic droplet formation could influence on viability and secretory functions of Langerhans islets. **Methods:** To investigate how the parameters of droplet generator influence on insulin secretion the hormone concentration were estimate during the encapsulated islets culture. Free, encapsulated islets were cultured for up to 10 days. The medium was changed every second day and the samples were taken and tested for insulin content. The islets in all tested groups were stained with dithizone and trypan blue before and after the culture. **Results:** In all groups was observed that 100% islets exhibited insulin production (red dye). In one of tested group the islets were stained with PI in 30% after 10-day culture. In the same group decline of insulin secretion was observed to compare with control. **Conclusions:** The viability and insulin secretion by encapsulated islets depends of parameters of process of their encapsulation.

O95 (EI0102)

Islets Transplantation in Diabetic Rats: Human Adipose Tissue-Derived Mesenchymal Stem Cells Enhance the Survival and Insulin Function of Cultivated Islets

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Objectives: Hypoxia plays a crucial role in pancreatic islet cell death during the culture period and in the first days after transplantation. Human mesenchymal stem cells (hMSCs) release antiapoptotic and angiogenic factors useful to protect islets during this period. The aim of this study was to evaluate the capacity of hMSCs isolated from adipose tissue (ADhMSCs) to protect islet viability and function in case of prolonged pre-transplant culture period. **Methods:** Rat pancreatic islets were cultivated for 72 hours in absence (group 1) or presence (group 2) of ADhMSCs (5×10^4 cells/100 islets). At the end of the culture period islet cell viability was evaluated by histological analysis with immunofluorescence. Subtherapeutical volumes of islets of the two groups (2000 ieq/Kg) were transplanted into the liver of streptozotocin-induced diabetic syngeneic rats (n = 5 per group). Rats were subjected to daily measure of glycemia and, two weeks after transplant, to intraperitoneal glucose tolerance testing (IPGTT). A morphological evaluation of graft was assessed by immunofluorescence over hepatic lobes harvested after sacrifice. **Results:** At the end of the culture period islets from group 2 showed a higher number of viable beta cells. Transplanted rats of group 2 presented a better glycemic control, detected by lower levels of blood glucose, than transplanted rats of group 1. IPGTT confirmed an enhanced islet function in group 2, which is resulted associated to an increased revascularization (larger number of lectin BS-1 positive cells) as highlighted by morphological analysis of the graft. **Conclusions:** ADhMSCs seems to be efficient in protecting islet cell viability during culture period. The use of ADhMSCs is potentially useful in preserving islet functionality in case of prolonged pretransplanted culture period.

New Biomaterials and Scaffolds—Symposium

O96 (EI0376)

Supermacroporous Cryopolymers for Tissue Regeneration

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Objectives: A range of porous synthetic and natural polymer hydrogels were produced using cryopolymerisation method and their potential as biomaterials for tissue regeneration was assessed. **Methods:** The cryopolymers of polyvinyl alcohol, poly-HEMA, fibrinogen, collagen and gelatin were synthesised in aqueous solutions at temperatures below 273 K. The ice crystals were used as a pore forming substance and the procedure led to formation of robust macroporous polymer structures capable of retaining their integrity upon multiple hydration-dehydration cycles. The pore structure of cryopolymers was characterised using cryo-NMR, confocal laser scanning microscopy, m-CT and SEM. Infiltration and proliferation of human dermal fibroblasts were used to study potential of cryopolymers as tissue scaffolds for wound healing. **Results:** The porous structure of cryopolymers was comprised of fully interconnected (super) macropores in the range of 50–200 mm, suitable for cell migration and proliferation. *In vitro* results in a human skin model followed by experiments using large animal model showed high healing properties of the cryopolymers, which were better than in control experiments using commercial materials. Depending on the application, cryopolymers can be made biodegradable or stable, which depends on degree of cross-linking and the nature of the polymer.

Conclusions: Cryopolymer based tissue scaffolds have shown high efficiency in wound healing and potential for internal organ regeneration.

O97 (EI0304)

Three-Dimensional Hydrogel Mimics Hierarchy and Size of Natural Fiber Bundles

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Objectives: In many natural tissues, fibrils align in parallel and closely pack into three-dimensional (3D) hierarchical bundles of fibers. These fibers provide tensile strength to various tissues such as heart or bone. Given their importance in tissue function, engineering these hierarchical features into materials is of great biomedical relevance. Numerous strategies for the development of a synthetic fiber bundle have been proposed, such as extrusion of polymers into aqueous solutions. However, most of the existing techniques fail to replicate the hierarchical architecture of these tissues. Thus, the aim of this work was to engineer hydrogel fibers that both mimic the natural architecture of the fiber bundles and enable the encapsulation of cells. **Methods:** Fiber bundles were fabricated by complexation between cationic chitosan (CHT) and anionic methacrylated gellan gum (MeGG) that occurred in a polydimethyl siloxane (PDMS) channel. Fibers were then collected and stabilized by photocrosslinking the MeGG. The resulting architecture of the fiber bundles was studied with atomic force (AFM), scanning electron (SEM), transmission electron microscopy (TEM) and confocal microscopy. A closer system to biological matrices was achieved by covalently incorporating the adhesive motif RGD in the MeGG backbone. **Results and Discussion:** Each bundle was approximately 100 μm in diameter and contained small fibers that were 1–5 μm in diameter. TEM revealed the structure at the nano-scale, exhibiting periodic gaps as in native collagen fiber bundles. Confocal microscopy of the hydrogel fiber bundles engineered with FITC-labeled CHT showed homogenous distribution of CHT throughout the fiber. Furthermore, encapsulated cardiac fibroblasts adhered to and spread along the fibril direction. **Conclusions:** This system combines polyelectrolyte complexation and fluidics technology to engineer hydrogel fibers that closely mimic the natural architecture of fiber bundles at different scales. Given its simplicity we envision that it may be beneficial for various tissue engineering and regenerative medicine applications.

O98 (EI0038)

Printing of a New Generation of Low Toxicity Polymers by Additive Manufacturing Technology for Bone Tissue Engineering

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Objectives: State of the art biocompatible and biodegradable poly(lactic acid) (PLA) has several disadvantages including bulk erosion mechanism, fast loss of mechanical properties, spontaneous release of acidic compounds and the inability to be structured by high resolution Additive Manufacturing Techniques (AMT). It was the aim of the current project to provide low toxic photopolymerizable formulations that can be printed by AMT to form 3D cellular scaffolds for bone tissue engineering with good biocompatibility and biodegradability. **Methods:** Currently, most of the used photopolymers for AMT are based on (meth)acrylates. Beside the considerable irritancy and sometimes cytotoxicity of acrylate-based monomers, the formation of high molecular polyacrylic acid through hydrolytic degradation of the polymer is another undesirable aspect of these materials when applied in the biomedical field. Therefore, photopolymers based on vinylesters and vinylcarbonates as polymerizable group, which give FDA approved, low molecular and water-soluble poly(vinyl alcohol) upon hydrolytic degradation, were evaluated. Several monomers based on different substrates were synthesized to examine their cytotoxicity, photoreactivity, mechanical properties and degradation behaviour. In vivo experiments of 3D parts were carried out in New Zealand White Rabbits. **Results:** In vitro studies with osteoblast-like cells, showed by far lower cytotoxicity than for their (meth)acrylate-based counterparts. By application of hydroxyapatite as filler mechanical properties already approached values from that of natural bone. The degradation behaviour of the new polymers can be easily tuned between several months and years. In-vivo studies showed excellent biocompatibility and osteoconductivity of the new materials. **Conclusions:** It has been proven that the new generation of polymers have outstanding properties for the application in the biomedical field. Beside low cytotoxicity of monomers, polymers and degradation products, the polymers have tuneable mechanical properties. Furthermore, the degradation behaviour can be tuned over a broad range and advantageous surface erosion mechanism (absence of acidic degradation products) can be seen.

O99 (EI0019)

Propensity-Matched Comparison of Drug-Eluting Stent Implantation and Coronary Artery Bypass Graft Surgery in Chronic Hemodialysis Patients

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Objectives: Cardiovascular disease is an important cause of death in patients with end-stage renal disease, with approximately 20% of cardiac deaths attributed to acute coronary syndrome. However, the optimal management of coronary artery disease in hemodialysis patients has not yet been determined. This study compared the outcomes of coronary artery bypass graft (CABG) surgery and drug-eluting stent (DES) implantation in hemodialysis patients. **Methods:** The study population consisted of chronic hemodialysis patients (dialysis duration >6 months) with coronary artery disease who underwent DES implantation or CABG at the Asan Medical Center (Seoul, Korea) between January 1, 1999, and February 28, 2006. Primary end points were major adverse cardiac and cerebral events (MACCE). Propensity score analysis was used to adjust selection bias and variable characteristics. **Results:** Of 110 chronic hemodialysis patients with coronary artery disease, 44 underwent DES implantation and 66 underwent CABG surgery. After propensity score adjustment, the incidence of MACCE was significantly higher in the DES than in the CABG group (HR, 2.791; 95% CI, 1.155–6.746; p = 0.023), but all-cause mortality did not differ between these groups (HR, 0.513; 95% CI, 0.095–2.773; p = 0.438). Kaplan-Meier analysis showed that MACCE-free survival was significantly longer in the CABG than in the DES group (log-Rank p = 0.023). **Conclusions:** We found that, compared with DES, CABG significantly reduced the incidence of MACCE and target vessel revascularization (TVR) in chronic hemodialysis patients.

Modeling of Cardiovascular and Pulmonary Function in Regard to Clinical Applications—Symposium

K18 (EI0440)

Physiological Modeling in Computational Design of Blood-Handling Devices

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Modeling and computational analysis play an increasingly important role in bioengineering, particularly in the design of implantable ventricular assist devices (VAD) and other blood-handling devices. Numerical simulation of blood flow and associated physiological phenomena has the potential to shorten the design cycle and give the designers important insights into causes of blood damage and suboptimal performance. A set of modeling techniques is presented which are based on stabilized space-time finite element formulation of the Navier-Stokes equations. Specific issues affecting shape optimization in this setting, such as parametrization of complex 3D surfaces and sensitivity to constitutive model selection, will be discussed. In order to obtain quantitative hemolysis prediction, cumulative tensor-based measures of strain experienced by individual blood cells must be developed; red blood cells under shear can be modelled as deforming droplets, and their deformation tracked along pathlines of the computed flow field. An alternative continuum-based approach is also under investigation. Another aspect of blood pump performance is related to platelet aggregation and thrombus formation. A three-species model for platelet aggregation is being developed based on a set of physiological experiments in collaboration with the Aachen University Clinic.

O100 (EI0034)

A Modified Sulfite Solution Measuring Oxygen Uptake of Oxygenators as an Alternative to Blood

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Objectives: Blood oxygenation is the main factor evaluating the efficacy of an oxygenator (artificial lung). In conventional calculation of oxygen transfer rate

(OTR) *in-vitro*, water and whole blood are used which consequences with inaccuracies and handling difficulties respectively. Therefore, a novel sulfite solution with modified concentrations of its components has been proposed which demonstrates a similar behavior to natural blood in case of oxygen uptake rate (OUR) and simulates its oxygenation. **Method**: The modified sulfite solution comprises 0.5 M sodium sulfite (acts as hemoglobin in oxygen uptake capability), 10^{-3} M cobalt sulfate (as catalyzer), 0.012 M phosphate buffer (Na_2HPO_4/NaH_2PO_4) and 10^{-5} M bromothymol blue (as color indicator). The solution's pH is adjusted at 8 with 30%wt sulfuric acid. To regulate the solution's OUR to that of natural blood, 32 ml of this solution should be diluted to 1000 ml. This solution is then tested with a hollow fiber membrane oxygenator (Medos AG, Germany). Using flow rates from 500 to 2800 [ml/min], oxygen uptake in this solution follows with a quick reduction of pH to 4, recorded by a sensitive pH sensor AZ8601, and is also indicated by color changes from blue to yellow. **Results**: The modified sulfite solution demonstrated similar maximum OUR (201 [mlO_2/L]) to natural blood, when all sodium sulfite reacted with O_2 and converted to sodium sulfate while pH remains constant around 4. Moreover, OTR in different flow rates shows an access of ±7.6% to blood in *in-vitro* tests e.g. with flow rate of 1000 [ml/min], the calculated OTR is 67 while the one of blood is 65 [mlO_2/min]. **Conclusions**: This modified sulfite solution can substitute with natural blood in OUR and OTR tests *in-vitro*. Therefore, it can be used as a reliable alternative to whole blood evaluating the performance of oxygenators in research and clinical applications.

O101 (EI0224)

A Numerical Study of Blood Flow Behavior in Blood Vessels with Emphasis on Thromboembolic Complications

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Objectives: Thrombotic events due to activation of platelets and plasmatic clotting factors can have fatal consequences. Especially patients who rely on vascular grafts or stents run an increased risk of developing thrombosis. Both the biomaterials and the flow properties in these conduits may trigger adverse platelet reactions. Blood flow conditions in arteries are characterized by computational fluid dynamics (CFD). Furthermore, a mathematical model to describe thrombocyte reactions is presented and validated with respect to experimental data in an in-vitro flow system modeling stenosed arterial flow. **Methods:** A continuum approach to modeling of platelet activation, adhesion and aggregation is used. The method is based on the advective and diffusive transport of resting and activated platelets and platelet-released agonists. Parallel computing resources allow to solve the equations on refined meshes. For the characterization of the role of different adhesive proteins, a shear flow configuration is used. Scanning electron and fluorescence microscopy are applied to evaluate platelet adhesion and visualize the binding proteins. **Results:** The nonpulsatile and pulsatile flow through healthy coronary, aortic and femoral arteries was computed as well as the flow through an occluded coronary artery. The dependence of thrombosis on the local flow conditions could be shown experimentally. The role of the binding proteins, e.g., fibrinogen and Von Willebrand Factor as a function of the local shear rate could be determined. **Conclusions:** Several arterial flow conditions and corresponding platelet behavior were studied both in-vitro and numerically. The presented simulations are in good agreement with experimental data.

O102 (EI0209)

Develpment of In Vitro Accelerated Fatigue Tester for Coronary Stent with a Function of Cyclic Bending

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Objectives: As proximal right coronary artery (RCA) is reported as one of the most frequent sites of stent fracture, we focused on fracture potentials of five drug-eluting-stents (DES) platforms using our in-vitro tester which can apply an in-vivo simulated cyclic-bend load. **Methods:** According to frontal coronary angiographic data of 63 patients, average bend angles of RCA between end-systole and end-diastole were analyzed to be 105° and 125°, respectively. The angle data classified in type-B referred to ACC/AHA guideline were used to fabricate a RCA silicone replica. Moreover, mechanical stiffness was adjusted to that of human coronary arteries. The accelerated durability tests were performed under the above cyclic bend environment. Cyclic bend-load was exerted 1200 times per minute to the stent installed in RCA replica filled with 37°C phosphate buffered saline. Fracture potentials of Cypher 316 L stainless steel (SS) stent (3.0 mm × 18 mm, Cordis), Taxus Express2 SS stent (3.0 mm × 20 mm, Boston Scientific), Liberte SS stent (3.0 mm × 20 mm, Boston Scientific), Driver cobalt alloy stent (3.0 × 18 mm, Medtronic), and Multi-Link Vision L605 cobalt chrome stent (3.0 mm × 18 mm, Abbott) were investigated (n = 6 each). All tests were conducted for 10 year-equivalent duration except for incidence of stent-separation. **Results:** No fracture was observed in Driver stents, however, Cypher stents were all fractured and complete separation of the mesh was occurred in 12 ± 6 days equivalent (n = 6). One Vision stent was completely-separated in 1020 days equivalent, while 5 Vision stents had no fracture. One Liberte stent had one single strut fracture, while other 5 Liberte stents had no fracture. Strut fractures with partly separation were observed in Taxus Express2 stents, which were started in 178 ± 310 days equivalent. **Conclusions:** A novel test platform to predict fracture potentials of clinically available stents was developed. The accelerated durability tester with a function of cyclic bending elucidated different incidences of stent fracture for five DES implanted at RCA region.

O103 (EI0182)

The Use of a Lumped Parameter Model to Optimize Biventricular Defibrillator Programming: Results Obtained by a Randomized and Prospective Study on 60 Patients

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Objectives: Biventricular defibrillator (BIV) implantation is a succesfull treatment for chronic heart failure despite 30% nonresponders, partly due to suboptimal atrioventricular (AV) and interventricular (VV) intervals programming. Aim of this work was to develop a numerical model (NM) for AV and VV optimization. **Methods:** A lumped parameter circulatory model was updated. Atria, ventricles, and septum were described by variable elastance models and their mechanical activity was related to ECG. BIV was modeled as an impulse generator driving heart chamber contraction. 60 patients were enrolled and randomized into two groups (A,B). All patients were studied by Echo, ECG, pressure measurement, six minute walking and Minnesota tests before BIV, 1, 3 and 6 months (fu1,3,6) later. In A, AV and VV were programmed and modified, if necessary, at each fu by NM optimizing hemodynamic variables. B was programmed by standard algorithm optimizing QRS. **Results:** There were no statistically significant differences between A and B at the baseline in QRS and left ventricular end systolic (LVes) and end diastolic volumes (LVed) (QRS:A:154.7 ± 39.5 ms;B:141.9 ± 39.5 ms;LVes:A:187.1 ± 92.7 ml;B:155.6 ± 57.6 ml;LVed:A: 246.1 ± 101,2;B:226.1 ± 67.1) and between simulated and measured data in A at the baseline and at fu6 ($p > 0,3$). At fu6, comparing A to B, it was observed:

- no statistically significant difference in QRS reduction ($p > 0,7$),
- a positive trend in Six minute and Minnesota tests ($p = ns$) and in ventricular remodeling(A:ΔLVes = −56.6 ml, ΔLVed = −53.7 ml;B:ΔLVes = −28.1 ml, ΔLVed = −22.45 ml, $p < 0,05$).

Conclusions: For each patient, the optimal AV and VV are different and can change at each follow up. A personalized and dynamic therapy based on the developed NM could improve patients outcome.

Artificial Kidney Dialysis Techniques—Symposium

K19 (EI0174)

Presence of Microbubbles in Haemodialysis. Physical Basis, Technical Considerations and Regulations

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Objectives: Microbubbles have been observed in the hemodialysis extracorporeal circuit (EC). The origin of these Microbubbles is still debated. The purpose of this work is the elimination of several possible causes of bubble production. **Methods:** Literature studies, calculations and photographic documentation **Results:** Degassing under the influence of negative pressure requires nucleation sites in blood. Numerous papers published between the 18th and the 21st century have demonstrated the absence of nucleation sites in venous (and arterial) blood. Sudden acceleration of the blood column may cause cavitation as known from metal heart valves. Acceleration caused by blood pump pulsations is more than a order of magnitude below the critical limit. Diffusion of air from the dialysate side can be excluded even if air is visible on the dialysate side because of the partial pressure gradient. The likely origin of air bubbles is air remaining in the EC including the dialyser after priming or leaks in the prepump part of the EC which is under negative pressure. Another source of air bubbles may be saline used for priming. **Conclusions:** The number of air bubbles reaching the patient from the EC can be reduced by careful priming. It is likely that priming by backfiltration or by online produced substitution fluid will result in considerably less air bubbles because dialysate is degassed and air bubbles remaining in dialyser pores may be expelled more efficiently during the priming phase.

O104 (EI0327)

Evaluation of Air Contamination Incidences and In Vitro Settings and Experiences of Microbubbles

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Objectives: During haemodialysis air infusion may occur. In Sweden incidents occurred using different systems. Air could be introduced into the blood stream from couplings where i.e., a negative pressure is present besides residual air. The aim was to find a method that could be used to verify presence and size of air contamination, such as microbubbles in dialysis extra corporal circuit. **Methods:** *Qualitative:* A dextran and albumin (D-A) solution was developed to simulate blood that enables study of microbubbles by visual inspection. *Quantitative:* Bubble detectors Hatteland CMD10, and EMX25 were used to count and verify size of bubbles. *Calibration:* The system was calibrated and validated using de-aired water and glass beads. *Measurement:* A set of blood lines was connected to a container with dextran-albumin solution that was recirculated in the system. A bubble detector probe was attached to the system. One variable at a time was changed; such as flow or venous chamber. Statistic: paired nonparametric statistics were used. **Results:** Visual inspection verified presence of microbubbles in venous lines. Calibration: There were no detected counts with de-aired water. There was a normal distributed count of glass beads with a range of a 9-graded scale (the size was calculated to be ≥45 µm at level 9 at 0 dB, down to 2,5 µm at 0 dB at grade 1). In vitro tests showed that the micro bubble distribution correlate to flow, dialysator and shape of venous chamber. **Conclusions:** The method using a D-A-solution is a fast qualitative method to get a view of bubble distribution in extra corporal systems. Together with a bubble detector and paired nonparametric statistics it is a robust and effective method for in vitro testing to evaluate and compare bubble exposure in extracorporal systems.

O105 (EI0228)

In Vitro Testing of Prevailing Materials and Initial Clinical Findings

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Objectives: During HD previous studies have shown that especially microbubbles of air may pass the air detector. These studies focused to analyse *in vitro* a, if the air trap of various producers may contribute to the presence of micro-embolic counts in the fluid that has passed the air trap detector. In addition another *in vitro* study analysed if the dialyzer by itself may contribute to these contaminations. In parallel a clinical study was performed to evaluate if findings during in vitro tests could be found to some extent during chronic haemodialysis. If such contamination would be present how frequent is this and would it be a greater risk at the start or the end of dialysis. **Methods:** An ultrasound probe was placed on the dialysis venous dialysis tube after the air detector and venous chamber (Hatteland, Norway). **Results:** The studies verified previous in vitro studies with micro-embolic counts that pass the air trap without inducing an alarm. Fewer embolic signals were detected in the in vitro studies when using a high level of the fluid in the air trap and when using a wet dialyzers. In the clinical studies a high counts were present both at the first period as well as the last period of haemodialysis. **Conclusions:** These studies verify the finding of embolic counts in various extent in various in vitro settings but also verify the presence of such counts in the dialysis tubes after the air trap in clinical routine haemodialysis. Further studies are warranted to clarify how to prevent patients from such problems and to clarify if the counts are clinically relevant.

O106 (EI0112)

A High Blood Level in the Venous Chamber and a Wet Stored (Gamma Sterilized) Dialyzer Help to Reduce Exposure for Microbubbles of Air

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Objectives: Twenty long-term haemodialysis patients with an age ranging from 42 to 80 years (mean age of 65 years) were investigated. There were eight women and twelve men. **Methods:** The patients were randomized to a cross-over of 3 modes of HD: Mode 1: F8HPS (Fresenius, steam sterilized) with a low blood level in the air chamber (FL); Mode 2: F8HPS with a high level (FH); Mode 3: Rexeed (Asahi Kasei Medical, gamma sterilized, wet stored dialyzer) with high blood level (RH). Microbubble measurements were continuous during 180 minutes of HD for all settings. The conditions were the same for each patient throughout the series. **Results:** A Multiple Poisson regression was used to test the effect of filter type on the amount of bubbles in the blood. There was a significant effect between different filter settings and bubbles detected in blood both for RH vs FL (OR 4.07, 95% CI 4.03–4.11, $p < 0.0001$) and FH (OR 1.18, 95% CI 1.17–1.19, $p < 0.0001$) and for FL vs FH (OR 0.290, 95% CI 0.288–0,293, $p < 0.0001$). This means that bubble exposure is least when using RH, more with FH and most with FL. **Conclusions:** During haemodialysis microbubbles of air develop in the blood circuit in the device. These microbubbles can pass the venous chamber and enter into the circulation of the patient. This study shows that using a high fluid level in the venous chamber results in a lower exposure for MB and by using wet stored dialyzers this exposure was even less. These cost limited measures may have an important impact to reduce the MB exposure to the patient.

O107 (EI0232)

Micro-Embolies of Air are deposited in the Organs in Haemodialysis Patients. A Case Report

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Objectives: HD patients (HDp) in comparison to peritoneal dialysis have and increased prevalence of pulmonary fibrosis and cerebral alterations. We previously showed that during HD the blood that passes the dialysis device gets loaded with microbubbles of air (MB) that are returned to the patient without inducing an alarm. These MBs can be detected as embolic signals by an ultrasound device. These embolic signals increased significantly during HD, both in the AV-fistula and in the carotid artery (Forsberg et al. 2010). Aim: To clarify if these signals are due to micro-embolies of air or blood or just artifacts we perform histopathology of autopsy material of HD patients (approved by the Ethical Committee). **Methods:** Our first results are from a 61-year old man who was on chronic haemodialysis for 5 years, due to diabetic nephropathy. During an episode of pulmonary edema, due to fluid overload, he was ultrafiltered. Within half an hour after start he suffered from a cardiac arrest and died. Autopsy verified the clinical findings. Tissue was fixed and stained using antibodies to C3, IgG, IgM and fibrinogen. **Results:** Microscopic investigation of the lungs, brain and heart verified the presence of micro-embolies of air that were surrounded by fibrin in these organs. The latter indicates that MBs were deposited before death occurred. **Conclusions:** Autopsy data show that micro-embolies of air enter the blood during HD in the dialysis device and are trapped in the lungs. In addition they pass the pulmonary capillaries and arterial part of the body and are dispersed throughout the whole body. These data strongly support that these MB cause micro-embolies and organ impairment and can be part of the bad prognoses found in HDp. Data also support the importance to reduce the extent of MBs in the dialysis circuit.

Natural-Based Polymeric Biomaterials and Composites for Regenerative Medicine—Symposium

O108 (EI0396)

Tissue Engineered Constructs for Periodontal Regeneration Based on Adipose Stem Cells and a Newly Designed Polymeric Scaffold

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Objectives: Periodontal disease, highly prevalent in human and canine species, is an inflammatory pathology which can result in tooth loss and in systemic implications. The current therapies are ineffective in avoiding the epithelium growth, bone resorption and ankilosis, which inhibit the formation of a functional periodontal ligament. Tissue engineering (TE) strategies, combining scaffolds and mesenchymal stem cells, has paved the way for new therapies. Our goal is to develop an innovative construct for periodontal regeneration, based on culturing adipose stem cells (ASCs) onto a bi-layered scaffold comprising a starch+poly(e-caprolactone) (SPCL) membrane, which acts as a guided tissue regeneration membrane, and a SPCL fibre mesh functionalized with osteoconductive silanol groups. **Methods:** The SPCL membrane was obtained by solvent casting and then combined with a wet-spun fibre mesh (WSFM) with/without silanol groups. Bi-layered scaffolds were characterized by scanning electron microscopy (SEM), tensile tests, Fourrier Transmission Infra-red (FTIR), and enzymatic degradation assays. Canine ASCs (cASCs) were obtained from subcutaneous adipose tissue harvested upon programmed surgeries. The proliferation of cASCs seeded/cultured onto the scaffold was studied by dsDNA quantification and SEM. Osteogenic differentiation on the WSFM was assessed by ALP quantification, real time RT-PCR (osteoblastic markers) and histology (Alizarin Red and Lévai Laczkó stainings). **Results:** SEM revealed a good adherence between the layers, roughness and fibres interconnection. FTIR confirmed the presence of Si-O-Si and Si-OH bonds in functionalized WSFM. Also, the scaffold exhibited a suitable mechanical properties and degradability to be applied in an *in vivo* environment. Culturing experiments showed that materials provide a good support for ASCs according to DNA increasing and SEM. ALP activity increasing until 21th day and also the calcium content revealing osteoconductivity and bioactivity. **Conclusions:**

This work showed that cASCs onto this SPCL bioactive scaffold are a promising TE approach to reach periodontal regeneration, namely, in its osseous component.

O109 (EI0439)

Natural-Based Photocrosslinkable Polyelectrolyte Complex Hydrogel: Development and Microfabrication

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Objectives: When two oppositely charged polyelectrolytes are mixed together, a physical hydrogel is formed through molecular interactions. These interactions are reversible and can be disrupted by changes in the ionic strength, pH or temperature. To surpass this, we used a photocrosslinkable polyelectrolyte that enabled the stabilization and microengineering of polyelectrolyte complex (PEC) hydrogel. **Methods:** Anionic methacrylated gellan gum (MeGG) was added to the cationic chitosan (CHT) solution in different ratios and exposed to UV light to form PEC hydrogels based on these natural polysaccharides. The chemistry of the surface and bulk of the hydrogel was analyzed by Fourier transform infrared spectroscopy (FTIR) and x-ray photoelectron spectroscopy (XPS). Transmission electron (TEM) and confocal microscopy showed the distribution of both polymers within the hydrogel. Microfabricated structures were produced by placing a photomask on top of the PEC. **Results and Discussion:** The initial electrostatic interactions that occurred upon contact between the two polymers did not allow for an instantaneous mixing. Upon capsule formation, the mixing of both polymers proceeded slowly. TEM and confocal of fluorescein-labeled CHT suggested the migration of CHT to the interior of the apparent MeGG capsule. FTIR and XPS chemically validated these findings. The photocrosslinkable feature of MeGG further enabled the formation of a number of micro-units with different shapes and sizes with viable encapsulated fibroblasts. **Conclusions:** We successfully fabricated stable photocrosslinkable PEC hydrogels using CHT and photocrosslinkable MeGG. This system is potentially useful for a variety of applications in regenerative medicine simply by changing the properties of the photocrosslinkable material and/or by changing the polymer charges, and therefore, the electrostatic interactions.

O110 (EI0138)

A Gelatin-Based, Cell-Free Scaffold for the Treatment of Articular Cartilage Defects—The Value of Early Tissue Reaction in an Animal Model

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Objective: Scaffolds are used to treat articular cartilage defects. We developed an oriented gelatin-based scaffold material, which imitates the fibre orientation in the middle zone of articular cartilage. In an experimental animal study the early phase of integration and regeneration of the scaffold combined with microdrilling was analysed and compared to microdrilling alone. **Methods**: According to a standardized procedure, two punch defects were generated in the medial and lateral condylus of the knee in five goats and treated with microdrilling. After randomization, half of the defects were filled with a scaffold. After 3 months the defect sites were explanted and analysed histologically by H&E and alcian blue staining. **Results**: Both groups showed regeneration of cartilaginous tissue with a mild predominance of hyaline cartilage in the scaffold group. The scaffold group revealed good regeneration of the subchondral bone. In the control group, necroses, and sequestration of the subchondral bone were evident. Furthermore, the scaffold group showed a good integration and regeneration from the edges of the defect, but not the control group. The superficial zones of the defects were free of blood vessels in the scaffold group, but not in the control group. **Discussion:** The blood vessel-free regeneration of hyaline cartilage was superior in the scaffold group compared to the control group. The pore orientation of the scaffolds might result in an optimized differentiation of inflowing cells and preferred formation of hyaline cartilage, since this orientation mimics the extracellular microenvironment of native cartilage. This could also be the reason for the lack of blood vessels in the scaffold group, since the scaffold-assisted differentiation into hyaline cartilage avoids the liberation of angiogenic factors. **Conclusion**: This study shows the impact of early time points analyses for a better understanding of scaffold integration regarding microenvironmental effects.

O111 (EI0072)

Sulfation of Glucosaminoglycan Effects on Protein Adsorption and Cell Adhesion

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Objectives: Protein- and cell-glucosaminoglycan (GAG) interactions have attracted great interests in the development of new therapeutics, for instance, tissue regeneration or cancer treatment. Most GAGs in nature possess sulfate groups, which contribute to multiple downstream signaling pathways. It has been reported that 6-O-sulfation of heparin is essential in FGF2 signal transduction, while 3-O-sulphation is required for anticoagulant activity.Here hyaluronic acid (HA), the only nonsulfated GAG existing in nature, has been studied as natural GAG and after sulfation. HA and sulfated HA surfaces were prepared through covalent bond between their oxidized derivatives and model surfaces, to study protein-GAG interaction and effects on cell-adhesion. **Methods:** Chemical structures of oxidized HA (ox-HA) and sulfated HA (ox-HAS) were indentified by FT-IR. Surface properties, namely, wettability, charges, morphology and average roughness, were examined by different physical methods. The immobilization of oxidized GAG and the adsorption of aggrecan were monitored by surface plasma resonance (SPR). Adhesion of human fibroblast on the different surfaces was studied by microscopy analyzing overall morphology, number and spreading area of cells. **Results:** FT-IR showed that the vicinal hydroxyl groups of HA and sulfated HA backbone were oxidized into dialdehydes, through which they could be immobilized on amino-silane modified substrata. The surface wettability and roughness increased after immobilization of oxidized molecules. In comparison with NH_2-modified surface, zeta-potentials of ox-HA and ox-HAS modified surfaces decreased to more negative values, which could be attributed to the presence of acidic dissociable surface groups. Compared to nonsulfated HA, the sulfated contributed to higher aggrecan adsorption and human fibroblasts adhesion. **Conclusions:** Sulfation of HA has a significant effect on protein- and cell-GAG interactions. Covalent immobilization of oxidized GAGs seems to be useful to engineer cellular attching behaviour,which might pave the way to tissue enginnering.

Partial Cardiac Support in Short and Long Term Application—Symposium

O112 (EI0117)

Hemodynamic Analysis of Efficacy of Pulsatile Perfusion During CPB with a New Centrifugal Pump

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Objectives: new models of centrifugal pumps are claimed to have better hemodynamic performance in pulsatile perfusion during CPB. Few data are available for hemodynamic evaluation of these pumps in-vivo, especially in high-risk groups as elderly patients. The study aims to compare hemodynamic effects of pulsatile versus nonpulsatile perfusion using MEDOS DeltaStream-DP3 centrifugal pump in patients over 75 years old. **Methods:** forty patients with sever aortic stenosis (mean age 80.7 ± 3.3, mean EuroScore 5.8 ± 1.4) undergoing AVR from 1.01.2010 to 31.01.2010 were prospectively randomized into pulsatile (n = 20 pts) and nonpulsatile groups (n = 20 pts). Pressure and flow curves were recorded simultaneously from external flow meters (TransonicHT110) and pressure monitor at 6 time points during CPB (at pre-oxygenator, post-oxygenator, aortic cannula and patients radial artery levels). Pulsatility was quantified in terms of energy equivalent pressure (EEP) and surplus hemodynamic energy (SHE). Hemodynamic indexes and clinical effects were monitored during 24 hours peri-operatively. **Results:** groups showed no difference in mean CPB time ($p = 0.98$), cross-clamp time ($p = 0.95$), mean perfusion flow ($p = 0.32$) and pressure ($p = 0.16$) values. In both groups the measured blood flow corresponded to the calculated one. Mean SHE generated at the outlet of the pump was113.5 ± 21.8 ergs/cm^3 with further progressive drop along the circuit until $5.3 + 1.9$ ergs/cm^3 calculated in the patient (4.7% from initial level). Pulsatile group showed lower vascular resistance during CPB ($p = 0.035$) and significant difference in SVR ($p = 0.04$) and PVR ($p = 0.02$) just after operation. Levels of SHE delivered to the patient correlated positively with urine output during CPB ($R = 0.34$, $p = 0.041$) and PVR after CPB ($R = 0.44$, $p = 0.015$). No differences between groups were found in pharmacologic support, transfusion rates, creatinine levels, respiratory indexes and intubation time. Longer ICU and hospital stay were related to severity of preoperative co-morbidities. **Conclusions**: pulsatile flow produced by MEDOS DeltaStream-DP3 centrifugal pump results in hemodynamic advantages and better tissue perfusion in high-risk patients.

O113 (EI0430)

Hemodynamics of a Valveless Counterpulsation Heart Assist Device: Particle Image Velocimetry and Wall-Particle Image Velocimetry

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NO AFFILIATIONS!!!

Objectives: The long-term application of counterpulsating devices (CPD) is limited by blood stagnation induced thrombus formation. A new CPD with 32 ml stroke volume was designed to prevent this cause of thrombus formation. Utilizing two measurement techniques, the blood washout behavior and wall shear stresses in the new design are obtained. A set of operating mode conditions, based on the time of membrane movement, were tested. **Methods:** The time resolved flow in the disk-shaped lumen of the valveless CPD was investigated with particle image velocimetry (PIV) and with wall-particle image velocimetry (wall-PIV). The PIV study is focused on the central plane of the blood pump, where maximal velocities are anticipated. The wall-PIV investigations consider potential areas of blood stagnation on the housing of the blood pump. **Results:** Flow investigations found that a tangentially designed CPD inlet port forms during a filling phase a strong, generally two-dimensional (2D) moving vortex fully filling the blood chamber. No regions of persistent blood stagnation or recirculation bubbles were observed. Shear stresses were moderate inside the blood chamber. The velocities in the lumen decay exponentially. Temporal vortex behavior was analyzed by its circulation frequencies. By comparison of wall shear rates and circulation frequencies the stagnation risk caused by different operation conditions was investigated. **Conclusions:** The time resolved flow in the CPD lumen shows good washing characteristics with no stable areas of blood stagnation. By setting a threshold for the shear rate a maximal hold time can be identified.

O114 (EI0104)

Mathematical Simulation of Clinical Scenarios During Support with Continuous Flow Left Ventricular Assist Devices (cf-LVAD)

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Objectives: Mathematical models can have important clinical diagnostic potential during mechanical circulatory support. In this research, we considered a mathematical model of the assisted heart and circulation with low complexity and established its potential to simulate multiple clinical scenarios of patients with cf-LVADs. **Methods:** The heart was modelled with the one-fiber model concept which relates cardiac pump function with myofiber mechanics. The circulation was modelled by lumped parameter models. A cf-LVAD was modelled based on pump characteristics measured ex vivo. The model parameters were adapted to simulate clinical scenarios during cf-LVAD support related to heart failure, myocardial tissue remodeling, aortic valve competence and obstruction of the cf-LVAD. **Results:** The simulation revealed that heart failure was characterized by increased ventricular dimensions, and decreased cardiac output and ejection fraction. The cf-LVAD led to decrease in dimensions and arterial pulsatility and caused permanent aortic valve closure. Improved contractility of the myocardium (cardiac recovery) led to improved arterial pulsatility and more aortic valve opening while, increase in myocardial tissue stiffness (atrophy) did not affect these parameters. Cf-LVAD flow increased with increase in aortic valve insufficiency while total cardiac output decreased. Aortic valve stenosis led to decreased cardiac output in the case of a pump stoppage. A large inflow or outflow obstruction area was necessary to cause a significant decrease in flow through the cf-LVAD. **Conclusions:** In conclusion, we present a mathematical model which simulates the effect of heart failure, myocardial remodeling aortic valve competence and cf-LVAD dysfunction. Such model can become an appropriate prognostic tool for the development of novel therapeutic strategies for patients supported by cf-LVADs.

O115 (EI0393)

Predicting Oxygenator Failure During VA-ECMO: Performance Indexes

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Objectives: Prolonged extracoproeal support for cardiac assist (VA-ECMO) is associated with a progressive impairment of oxygenators (ML) performance. Once MLs became inefficient change-out (ChO) is mandatory and should be performed electively to decrease morbidity due to circulatory lack of assistance during ChO procedure. Up to now ML are changed according to clinical nonstandardized criteria. We analyzed effectiveness of variations in resistances to blood flow (BFR), O_2 transfer capability (TO_2), D-dimer and a combination of BFR-TO_2 (performance index, PI = BFR*TO_2) in predicting ChO need. **Methods:** We rewieved 2008–2010 clinical data of all the patients treated with VA-ECMO in the cardiac ICU of San Gerardo University Hospital; ECMO technology was uniform (centrifugal pump Jostra Rotaflow, PLS oxygenator, Maquet, Germany). Exclusion criteria were: preexistent disease affecting coagulation, fibrinolytic therapy, VA-ECMO duration <6 days. Decision for ChO was made according to clinical judgment. ROC analysis was performed on day 2 and day 1 data after setting the last day of ML that did not need ChO at the average day of ChO. **Results:** 31 patients were enrolled, (54% males, 64 ± 15 years old) accounting for 35 ML (10 ChO). ChO occurred on day 6.4 ± 3.8. Best predictive parameters resulted PI (average ± SD 993 ± 541 and 1025 ± 451 at baseline and ChO day respectively in CG) and the percent variation of D-dimer: AUC were respectively 0.80 (78% sensibility, 88% specificity, cut-off 1460) and 0.88 (88% sensibility, 87% specificity, cut-off 243%) at the day-1, and 0.66 (80% sensibility, 56% specificity, cut-off 1046) and 0.81 (89% sensibility, 75% specificity, cut-off 119%) at day-2. No correlation was found between D-dimer and PI variations. **Conclusions:** An index combining TO_2 and BFR, and increase in D-dimer may predict oxygenator failure with high sensibility and specificity.

Artificial Organs—Practical Applications—General Session

O116 (EI0337)

An Innovative Serum-Free Culture System for Expansion and Osteogenic Differentiation of Adipose and Amniotic-Derived Stem Cells

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Objectives: Increase of life expectancy in the last decades resulted in more frequent aging problems, especially organ failure and tissue malfunctioning which cause reduction of life's quality. Present methodologies are still insufficient, therefore the ultimate solution may rely on the development of appropriate regenerative medicine strategies. Underlying these goals there is the need for expansion of stem cells (SC) which are the primary players of tissue regeneration. Although several studies have reported the use of MSCs from adipose tissue, amniotic fluid (AF) and placenta in clinical settings, major hurdles for their clinical use are related to the need of animal supplements, and the small number of cells that can be isolated despite the large number of cells needed. Thus, our goal is to evaluate the proliferative and osteogenic potential of adipose- and AF-derived stem cells expanded in an enhanced serum-free culture system. **Methods:** We isolated hAFSCs from the supernatant of day 6 cultures of amniotic fluid obtained from amniocentesis, and adipose stem cells (hASCs) from liposuctions. Cells were expanded in alpha-MEM supplemented with IGF-I and -II, bFGF and PDGF-BB, and compared to commercially available MesenCult-XF. Cell expansion was evaluated by cell counts and DNA quantification. Expanded cells "stemness" and osteogenic potential were evaluated by flow cytometry and qRT-PCR. **Results:** It was observed a progressive increase in cell number with the culturing time. Cell proliferation is favored by the increase in initial cell density, demonstrating the importance of cell-cell interactions. This effect is more pronounced in ASCs cultures. Increasing the cell passage, we observed a loss of proliferative potential independently of serum-concentration in the culture medium, for the SC of both origins. **Conclusions:** With this work we expect to contribute to the paving of the way to produce a culture system envisioning its use in regenerative medicine and broader clinical applications.

O117 (EI0275)

The Next-Generation Nanocomposite Materials for the Development of Tissue Engineered Organs and Tissues

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Objectives: Persisting organ shortages concurring with exponentially rising demands for donor tissues and organs have sparked a biotechnological race for the synthesis of artificial alternatives. Recently, fundamental advancements within the field of tissue engineering have furthered the potential for developing optimised bio-artificial substitutes. This comprises the growth of new tissue in a biological or synthetic scaffold within a bioreactor. In our laboratories, we have developed and patented a family of new generation nanocomposite polymers based on polyhedral oligomeric silsesqioxane (POSS) integrated poly(carbonate) urea-urethane (PCU) (nonbiodegradable) as well as the biodegradable pendant POSS integrated poly(caprolactone) urea-urethane (PCL) for the creation of 3-dimensional scaffolds for surgical applications. **Methods:** Here, we present the characterization of our nanocomposite polymers including methods of fabrication, relevance of surface nanotopography in relation to biocompatibility and cell attachment, cellular integration and viability as well as their proliferative capacity. Polymers were fabricated using electrospinning as well as ultrasonic atomization spraying techniques. Utilising

a tissue engineering approach, 3-dimensional polymeric scaffolds were created, characterised and integrated with various cell types including endothelial progenitor cells (EPCs) and adipose-derived stem cells (ADSCs). **Results:** Integration of the POSS nanocages into the polymeric scaffolds conferred material biostability, anti-inflammatory and anti-thrombogenic properties and changed the surface nanotopography of the scaffold to create a more favourable extracellular environment for cells seeded onto it. EPCs and ADSCs were successfully grown on the nanocomposite scaffolds and showed viability as well as proliferative capacity. **Conclusions:** There remains an un-met clinical need for effective scaffolds for tissue engineering biological substitutes. We have succeeded in developing new generation nanocomposite materials based on smart, bioactive, nanostructured materials to develop new types of tissue engineering scaffolds for the regeneration of tissues and organs.

O118 (EI0333)

Intracellular Methylprednisolone Release to Glial Cells Using an Engineered Dendrimer Nanoparticle System

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Objectives: SCI therapies do not present effective solutions due to the lack of efficiency of the drugs used. One possible approach to circumvent this could be the use of cell-specific intracellular drug delivery systems that could act on the neuroprotection and regeneration of the lesion. Thus, we are proposing the use of a dendrimer-based nanoparticle system composed of a polyamidoamine (PAMAM) core and grafted with carboxymethylchitosan (CMCht). These nanoparticles will be loaded with methylprednisolone (MP), an anti-inflammatory corticosteroid that would be carried to the site of lesion. **Methods:** CMCht/PAMAM dendrimer nanoparticles (NPs) were synthesized and MP was incorporated. MP-loaded NPs were labeled with fluorescein isothiocianate (FITC) to evaluate internalization and intracellular trafficking. Particle size and zeta potential analysis were performed using the Zetasizer equipment. MP release profile was assessed by HPLC in two different buffer solutions (pH 5.0 and pH 7.4). Finally, glial and microglial cultures were established to evaluate the NPs behavior when in contact with these cells. **Results:** MP-loaded NPs possess diameters around 109 nm and are stable at physiological pH. They do not affect glial cells' viability or proliferation. Also they were easily internalized by all CNS cell types reaching 100% internalization 24 hours after NPs addition. MP release profile revealed an initial burst within the first 24 hours followed by a sustained release for periods up to 14 days. The anti-inflammatory profile of these NPs was assessed in microglial cell cultures. The MP released from the NPs induced a significant decrease on microglial viability. **Conclusions:** These results indicate that these dendrimer-based NPs have potential to be used as modulators of the inflammatory events in SCI sites. Additionally, they are excellent intracellular delivery carriers, entering the cells at high rates and releasing the incorporated drug within its cytoplasmic compartment, and allowing its action to be carried out.

O119 (EI0106)

Effects of Unfractioned Heparin and Low-Molecular-Weight Heparin on Osteoprotegerin and RANKL Plasma Levels in Hemodialysis Patients

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Objectives: This randomized crossover study investigated the effects of unfractioned heparin (UFH) and low-molecular-weight heparin (LMWH) on intra- and post-dialytic blood levels of osteoprotegerin (OPG), receptor activator of nuclear factor kappa B ligand (RANKL) and inflammatory cytokines in dialysis patients. **Methods:** We selected 40 patients on hemodialysis for 12 months. UFH or LMWH was randomly assigned and maintained for 1 month, and then each patient was switched to the other form of heparin. In the midweek session, we determined anti-Xa activity, OPG, RANKL, IL-1β, IL-6 and TNF-α values before heparin administration and after 15 min, 4, 8 and 24 h (T0, T1, T2, T3 and T4 respectively). **Results:** A highly significant ($P < 0.001$) increase in anti-Xa activity was detected at T1, regardless of the type of heparin (UFH or LMWH), as confirmed in the comparison of T0 vs T1 using one-way ANOVA. Moreover, with both heparins, significant differences were found at T1 vs T2 (both $P < 0.001$) and at T2 vs T3 ($P = 0.0003$ with UFH; $P < 0.001$ with LMWH). Conversely, the difference in anti-Xa activity at T3 vs T4 was still significant with UFH ($P = 0.0186$) but not with LMWH. Anti-Xa activity at T4 vs T0 was not significant different either with UFH or LMWH, indicating that 24 h after heparin infusion, it returned back to pre-infusion values. Changes in OPG levels over time, regardless of the type of heparin, showed an increase in circulating OPG with a zenith at T1, and a return back to the baseline levels within the 24 hours postinfusion. Significant differences in OPG blood levels was shown at T0 vs T1 with both UFH ($P = 0.0112$) and LMWH ($P = 0.0288$). **Conclusions:** These results suggest that heparin-regulated cyclic increases of OPG might play a role in vascular pathology of hemodialysis patients.

O120 (EI0083)

Accelerated Removal of Tnf Within a Hemoadsorption Device Used to Treat Sepsis

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Objectives: Sepsis, systemic inflammation due to infection, is characterized by high concentrations of inflammatory cytokines in the circulating blood. Extracorporeal blood purification using biocompatible, microporous sorbent beads has been shown by us to decrease circulating cytokine levels and increase survival time in septic rats. The objective of this study was to develop novel techniques to accelerate capture of TNF, a large pro-inflammatory cytokine, within the hemoadsorption device. We propose surface modified sorbent beads capable of dissociating trimeric TNF into monomeric form, thereby accelerating removal of TNF from the circulating blood. **Methods:** TNF in horse serum was incubated with 10% DMSO to promote deoligomerization of trimeric TNF. Size exclusion chromatography was used to determine effects of DMSO incubation on TNF molecular size. Effects of TNF deoligomerization on capture was evaluated using an *in vitro* recirculation loop. TNF (~1 ng/ml) was spiked into 8 ml horse serum, and recirculated through a column packed with 1.5 g sorbent beads. Aliquots were periodically sampled from the reservoir, and TNF concentration was quantified using ELISA. **Results:** Native TNF eluted as a 34 kD oligomer using size exclusion chromatography. Incubation with 10% DMSO dissociated the TNF oligomer and resulted in elution of a 10 kD monomeric form. TNF capture from serum within the device was significantly accelerated after DMSO incubation, compared to native TNF capture (95% removal vs. 64% removal after 4 hours, respectively). **Conclusions:** TNF capture within the hemoadsorption device is slow due to the large size of trimeric TNF. TNF removal was significantly accelerated by dissociating oligomeric TNF, thereby allowing fast diffusion of monomeric TNF into the sorbent pores, and enhancing overall removal of bioactive TNF. We are developing surface modified sorbent beads to locally dissociate TNF within the device, as a novel method for increasing TNF removal rates while retaining an optimal sorbent pore size for albumin exclusion.

O121 (EI0240)

Epoetin-α/Darbepoetin-α Switch in Hemodialysis Patients: Trial to Evaluate the Treatment Efficacy in Achieving Hemoglobin Target Levels and Optimization of Cost/Efficacy Ratio

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Objectives: Recombinant human erythropoietins (rHuEPOs) as epoetin-α and epoetin-β are effective in the treatment of renal anemia. Darbepoetin-α differs from rHuEPOs for longer serum half-life and greater erythropoietic activity. Aim of the study was to compare the efficacy of the switch and associated costs from epoetin-α to darbepoetin-α for the treatment of anaemia in hemodialysis using a new dose conversion ratio (DCR). **Methods:** 78 patients were treated. The visits were defined as follows: T1)start of epoetin-α therapy; T2)6 months after T1, switch to darbepoetin-α; T3)1 month after T2, evaluations after the switch; T4)6 months from T3, final evaluations. The DCR generally used to change epoetin-α to darbepoetin-α is based on the protein mass equivalence between molecules (200:1). According to our routine clinical practice of erythropoiesis stimulating agents (ESA) management, the initial DCR was set up to 254:1. **Results:** At T2 epoetin-α mean dose was 13,717.95 IU/week (equivalent to a mean dose of 53.91 μg/week of darbepoetin-α), whereas the mean darbepoetin-α dose at T3 was markedly reduced($p < 0.05$) to 50.26 μg/week and 44.36 μg/week at T4. The mean Hb values measured at T1, T2 and T3 were 11.05 g/dL, 11.19 g/dL and 11.4 g/dL, displaying a progressive increase from T1 to T3 and a significant increase between the concentrations observed at T2 and T3($p < 0.01$). An increase in the mean Hb corresponds to a decrease of the mean weekly dose of administered darbepoetin-α($p < 0.05$). The proportions of patients reaching Hb target ≥11 and ≥12 g/dL were higher after darbepoetin-α (65.7%vs29.3%) compared to epoetin-α (57.4%vs25.2%). DCR was 335.37. **Conclusions:** Both ESA are effective in renal anemia. However, while epoetin-α group showed a modest increase of hemoglobin levels, darbepoetin treated patients had a better improvement. Our results suggest that, in order to maintain stable haemoglobin levels, the use of a DCR superior to 250:1 is required. Clinical benefits apart, darbepoietin-α can provide economical benefits.

Nondestructive Techniques to Monitor 3D in Vitro Tissue Engineering Constructs—Symposium

K20 (EI0095)

Optical Techniques for Monitoring 3D Tissue Constructs

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Objectives: Optical techniques offer potential for functional imaging over a wide range of cell scales from subcellular through to whole organ. An overview of optical techniques that are currently being applied to monitoring of 3D tissue constructs will be provided. The focus will then fall on (i) the use of a combined optics plus ultrasound system for monitoring tissue growth in scaffolds and (ii) the application of machine vision methods to monitoring cell aggregation in rotary cell culture systems. **Methods:** An overview of nonlinear microscopy and optical coherence tomography will be provided. An ultrasound modulated optical tomography system has been constructed to image tissue constructs in 3 dimensions which involves laser illumination and an ultrasound transducer. Light passing through the focus becomes modulated at the ultrasound frequency allowing the light to be 'tagged' and the effects of light scattering to be reduced. A machine vision and imaging processing system has been developed to automatically monitor cell aggregation. **Results:** Key recent results from nonlinear microscopy and optical coherence tomography will be highlighted. Images of absorbing and fluorescent targets embedded in tissue scaffolds (gels and foamed scaffolds) will be presented. Images of the cell aggregation process in a rotary cell culture system and properties extracted from the images (e.g. number of aggregates v time) will be shown. **Conclusions:** Optical techniques can provide functional imaging and monitoring over a wide range of sizes, from the subcellular to whole organs. This offers the potential for both better research tools and in industrial scale up of artificial organs.

O122 (EI0389)

Non-Destructive Quality Control for Islet Transplantation Using Raman Spectroscopy

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Objectives: Type 1 diabetes patients with poorly controllable glucose levels, can be treated by intrahepatic transplantation of donor islets of Langerhans. Before islets are transplanted into the patient, their quality needs to be assessed. Current quality control requires fixation and labeling and does not allow time-lapse studies on the same tissue. In this study we explore the feasibility of using Raman spectroscopy to perform functional studies on pancreatic islets and to monitor their quality over time. **Methods:** Raman measurements were performed using a home-built confocal Raman spectrometer. A krypton ion laser emitting at 647.1 nm and a 40× air objective were used to excite the sample, and the Raman scattered photons were collected and dispersed on an air-cooled electron-multiplying charge-coupled device. **Results:** We first used Raman spectroscopy to measure purified insulin and glucagon, the two main hormones produced by pancreatic islets. Raman bands at 520 and 640 cm^{-1} can be assigned to cysteine and tyrosine, amino acids that are present in insulin. Tryptophan, one of the building blocks of glucagon, causes specific bands at 759 and 1552 cm^{-1}. These bands can be used as markers for the identification of beta and alpha cells in islet preparations. We subsequently measured human islets and compared their spectral characteristics to those of insulin and glucagon. Tryptophan-specific Raman bands were observed in the islets spectrum, suggestive for the presence of glucagon-producing alpha cells. Bands suggestive for insulin were not observed in the average islet spectrum, possibly because insulin is a weaker Raman scatterer. High resolution local measurements on individual islet cells are currently performed to identify the presence of insulin-vesicles inside these cells. **Conclusions:** Our data provides the first steps towards a nondestructive and label-free method to study pancreatic islet quality before transplantation in type 1 diabetes patients.

O123 (EI0039)

The Study of Optical Properties and Proteoglycan Content of Native and Tissue Engineering Tendons by Ps-Oct

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Objective: Tendons are load-bearing collagenous tissues consisting mainly of type I collagen and various proteoglycans (PGs). It is widely accepted that highly orientated collagen fibers in tendons play a critical role for transferring tensile stress and demonstrate birefringence optical properties. Although PG's are the essential components of the tendon extracellular matrix, the influence of proteoglycans on its optical properties is yet to be fully elucidated. On the other hand, it is not fully clarified what is the key factor which regulates tendon optical properties during its regeneration. The objective of this study is using a nondestructive optical image technique to study the effect of PG content on collagen fibril organization during PG extraction in terms of birefringence alteration; also identify the essential parameters which can alter tendon's spatial organization manifesting as birefringence alteration. **Methods:** Fresh chicken leg tendons were dissected and used within 48 hours of dissection. The total proteoglycan was extracted by 4 M/2 M guanidine hydrochloride. The acellular engineered tendon made from polylactic acid nanofiber and collagen hydrogel were fabricated and the density of nanofiber and collagen hydrogel were varied. A bench-top fibre based time-domain polarization sensitive optical coherence tomography (PS-OCT) system was used to acquire the cross-section birefringence images during the PG extraction up to two hours. The PS-OCT images of acellular tendons were taken at different fabrication conditions. **Results and Discussion:** Extraction of GAG resulted in distortion of birefringence bands in native tendons. Higher concentration and longer extraction time led to destruct the birefringence bands rapidly and completely. Using low collagen density hydrogel did not generate acelular tendon with birefringence bands no matter the presence of nanofiber or not. **Conclusions:** The nondestructive imaging technique, PS-OCT, is a reliable and simple monitoring tool for studying spatial structure of highly organized tendon tissue for quality control or regeneration regulation.

O124 (EI0295)

Novel Approach for in Vivo Non-Invasive Vascular Graft Compliance Measurement

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Objectives: Compliance mismatch between a vascular prosthesis and the adjacent native artery has been related to neointimal hyperplasia leading to graft failure. A significant amount of research is currently being led in the field of degradable vascular grafts and proper characterization of mechanical properties *in vitro* is customary. However, mechanical properties of these structures *in vivo* may significantly evolve due to tissue remodeling and material degradation, but these changes are generally poorly monitored and described. The aim of this study is to report a new noninvasive method of *in vivo* vascular graft compliance measurement in the rat model. **Methods:** Five male Sprague Dawley rats (275 g) received an infrarenal abdominal aorta replacement with a poly(ε-caprolactone) (PCL) electrospun prosthesis. High resolution ultrasonography (VEVO 770, VisualSonics Inc., CAN) was performed to assess systolic and diastolic internal diameters of implants and adjacent abdominal aortas. Noninvasive arterial systolic and diastolic blood pressure measurements (CODA Monitor, Kent Scientific Corp., USA) were simultaneously carried out. This data was used to calculate *in vivo* compliance. **Results:** The average compliance of implanted PCL grafts was 9.4 ± 0.5%/100 mm Hg. Proximal and distal native aortas had an average compliance of 26.38 ± 4.2%/100 mm Hg and 21.5 ± 4.5%/100 mm Hg respectively. The compliance of the native aorta was significantly higher than PCL grafts ($p = 0.0014$) revealing a compliance mismatch. **Conclusions:** A reliable measurement of native and prosthetic vascular compliance *in vivo* is feasible in small animal models. This new method can provide time-related insight on compliance changes of biodegradable vascular grafts and lead to better design of next generation vascular grafts.

O125 (EI0004)

Development of an Implatable Small Camera for Angiogenesis

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Objectives: Angiogenesis is essential for successful tissue-engineered artificial organs and regenerative medicine. However, the mechanisms and conditions required for angiogenesis have not been disclosed yet. In this study, we monitored growth of blood vessels *in vivo* to study the angiogenesis mechanism. **Materials and Methods:** A miniaturized video camera system integrated with a scaffold for blood vessels and tissue induction that is implantable into an animal body was developed. A polyglycolic acid sheet of 0.3 mm thickness was used as a scaffold. The camera was implanted with the scaffold, and we observed angiogenesis into the scaffold. **Results:** We can observed tissue induction to the scaffold. It was started from second week and it was covered the entire area by the tissue about 10 weeks. Vigorous angiogenesis was observed at the front region of tissue induction resulting dense distribution of capillary vessels and red blood cells.

Stent and Vascular Prosthesis—General Session

O126 (EI0408)

In-Vivo Evaluation of Electrospun, Biodegradable and Non-Degradable Elastomeric Vascular Grafts

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Objectives: Biodegradable polymer scaffolds for tissue engineering applications have been the focus of intensive research during the last years. Polyurethanes (PU) are ideal candidates for vascular substitutes due to their excellent mechanical properties. In this study we evaluated the in-vivo behaviour of degradable and nondegradable, electrospun polyurethane grafts in a rat model. **Methods:** Biodegradable (aliphatic PU, n = 8) and nondegradable (Pellethan 2563–80 A, n = 8) mesh grafts were fabricated by electrospinning (void fraction 80%, fiber diameter 0.8–1.5 µm, inner diameter 1.5 mm, wall thickness 100 µm, length 15 mm). E-ptfe grafts (n = 8, inner diameter 1.5, wall thickness 100 µm, 25 µm IND, length 15 mm) were used as controls. Grafts were implanted into the aorta of 24 rats and analyzed after 6 months by biomechanical analysis, immunohistochemistry, scanning and transmission electron microscopy and morphometric techniques. **Results:** All grafts showed no signs of thrombus formation and aneurysmal dilatation. Both PU mesh grafts showed significantly increased transmural cellular ingrowth and complete endothelial coverage. More than 50% of the original wall of the aliphatic PU grafts was replaced by vascular specific tissue. Neointima formation was increased in eptfe and in aliphatic PU grafts. Tensile tests revealed a loss of strength of 12% for the nondegradable PU graft and of 45% for the degradable PU conduit. **Conclusions:** Both PU mesh grafts have the potential to attract vascular specific cells. It has to be evaluated in long-term studies if balanced tissue remodeling may compensate the loss of mechanical strength of the biodegradable implants.

O127 (EI0041)

In-Body Tissue Engineered and Completely Autologous Aortic Valved Conduit (Biovalve) In a Goat Model

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Objectives: Autologous valved conduits without any artificial materials (BIOVALVE) were developed, using simple, safe, and economical in-body tissue engineering. In this study, the potential of the BIOVALVE as an aortic valve was investigated in a goat model. **Methods:** BIOVALVEs were prepared by 2-month embedding of the molds, assembled using 2 types of plastic rods, in the subcutaneous spaces of goats. After removing the molds, BIOVALVEs with 3 leaflets and sinus of Valsalva similar to those of native aortic valves consisted of perfectly autologous connective tissues. BIOVALVEs were implanted as aortic valves using the apico-aortic bypass method for 2 months. **Results:** Postoperative echocardiography and angiography showed smooth movement of the leaflets with little regurgitation under systemic circulation. Histological examination afer 2 months showed that α-SMA–positive cells appeared significantly with rich angiogenesis in the conduit and expanded toward the tip of the leaflet. At the sinus portions, marked elastic fibers were formed. The luminal surface was covered with thin pseudointima. **Conclusions:** Completely autologous BIOVALVEs with robust and elastic characteristics satisfied the higher requirements of systemic circulation in goats for 2 months.

O128 (EI0189)

Development of Tailor-Made Semilunar Transcatheter Heart Valves

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Objectives: Transcatheter heart valve implantation is a young and innovative technology offering a novel form of treatment to patients previously classified as "no-option patients," due to comorbidities preventing open heart surgery associated with surgical valve replacement. Initial clinical results of transcatheter aortic and pulmonary valve replacement demonstrate promising results but also reveal the need for improvement. Factors which require technological development are repositionability and anchoring of prostheses to prevent device migration. Since suturing the valve into place is not possible in a transcatheter implantation, anchoring must be achieved through optimal anatomical fit. **Methods:** The diversity of pathological morphologies of the left and right ventricular outflow tract in patients indicated for transcatheter heart valve surgery leads to poor anatomical fit, paravalvular leakage or prosthesis migration. This study presents a new strategy for designing and manufacturing transcatheter heart valves in a system referred to as the "custom assembly kit." In this system, the prosthesis is composed of up to three components, one base valve element, having three leaflets, as well as proximal and distal anchoring elements. These three components can be assembled according to the anatomicy of a specific patient anatomy and provide optimized anchoring to prevent valve migration. **Results:** CAD designs and early prototypes of the concept demonstrate its feasibility. Excellent anatomical fit of the device is achieved by offering different diameters and geometries for the anchoring elements to connect to the base valve element. The assembled device offers the best possible prosthesis for a given pathological valve and outflow tract morphology. The design and development of this device are performed in close collaboration between biomedical engineers and cardiologists to ensure optimal mechanical durability, longevity and anatomical fit. **Conclusions:** This system represents the next step in transcatheter heart valve technology and will be further developed to form a marketable device.

O129 (EI0163)

The Sorin Freedom Stentless Pericardial Valve: A Valid Benchmark for Current Percutaneous Devices

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Objectives: Transcatheter aortic valve implantation (TAVI) has emerged as an alternative to aortic valve replacement(AVR) for severe aortic stenosis. The majority of TAVI systems currently available incorporate pericardial stentless bioprostheses with only short follow-up data available. Therefore information on long-term results of AVR with a stentless pericardial valve, such as the Sorin Freedom (SF), represents a valid benchmark. In this study we report the clinical and hemodynamic performance of SF with a 10-year follow-up. **Methods:** from January 2000 to December 2004, 78 patients, mean age5.6 ± 5.8 years, underwent AVR with SF. Sixteen (20.5%) were in NYHA class IV, 18 (23.1%) in III, 44(56.4%) in class I or II. Mean ejection fraction (LVEF) was 58.11 ± 11.16%. Echocardiographic evaluation was performed at 3, 12 months and yearly thereafter assessing effective orifice area (EOA), gradients (PG) and regression of left ventricular mass index (LVMi). **Results:** there was 1 operative death (1.2%). A total of 77 patients were discharged and followed for total follow-up of 5602 months (mean,70 ± 25 months). There were 24 late deaths with an actuarial survival of 56 ± 8.8% at 10 years. Three patients were reoperated, with a freedom from reoperation of 95 ± 3% at 10 years, because of structural deterioration, endocarditis and dilatation of sinotubular junction, respectively. At last clinical control 47 patients (90%) were in NYHA class I or II and 5 patients (9%) were in NYHA class III. Mean EOA varied from 1.8 ± 0.8 cm^2 for valve size21 to 2.3 ± 0.6 cm^2 for size27 and mean PG varied from 22 ± 9 mm Hg for valve size 21 to 13 ± 4 mm Hg for size 27. LVMi decreased from 182.9 ± 39.6 gm/m^2 to 142.1 ± 42.6 gm/m^2 ($p < 0.001$). **Conclusions:** SF stentless bioprosthesis has provided good results in terms of valve durability and freedom from valve-related complications with excellent hemodynamic performance at 10-year follow-up. These data represent important reference point against which performance of current TAVI systems must be compared.

O130 (EI0249)

A Novel Approach for The Development of Polyurethane Valve Leaflets

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Objectives: Modern heart valve prostheses are either mechanical disk valves or biological prostheses with porcine or bovine pericardial leaflets. Mechanical heart valves demonstrate good durability, but require lifelong anticoagulation due to thrombosis and hemolysis. Biological prostheses do not require anticoagulation, but are prone to calcification and degeneration. The use of polyurethane as a leaflet material allows the combination of durable synthetic materials with the flow characteristics of tricuspid biological prostheses. To make use of these advantages, a reproducible manufacturing process is required. In this study a novel development approach for polyurethane valve leaflets, manufactured from polyurethane tubes, is presented. **Methods:** In order to manufacture polyurethane heart valves with high durability, low residual stresses are required. This is initially achieved by molding polymer films from medical grade polyurethane and winding these to tubes. The edges of the films are connected using solvent. The manufactured tubes were then mounted on polymer stents with edges representing the form of the leaflet commissures of natural tricuspid valves. In order to improve valve kinematics, the leaflets were clamped at the commissure tips and joined. The resulting heart valve prostheses were tested in a accelerated wear tester. First endurance tests were performed with distilled water, at a testing frequency of 10 Hz and physiological pressures. With this setup, 6 samples were tested to failure. **Results:** All tube valve samples showed good closing behavior and pressure response. The tested valves endured up to 12 Mio. cycles before failing by

tearing along the leaflet edges. Leaflet fracture resulted in pressure loss without fragmentation. **Conclusions:** Artificial heart valve leaflets manufactured from polyurethane tubes give an encouraging perspective for future heart valve prostheses. Further investigation is focused on seamlessly manufactured tubes, optimized pre-forming of the valves and different polyurethane material grades.

O131 (EI0020)

The Biostent—Novel Concept of a Viable Stent Structure

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Objectives: The percutaneous stent angioplasty of peripheral vessels is well established as clinical routine. Unfortunately the patency rates of small-caliber grafts (<6 mm) are still unsatisfying, especially in the lower limb region. The aim of a novel BioStent concept is to overcome vessel occlusion due to both a total exclusion of the atherosclerotic plaques from the blood stream and an intact, functional active endothelial cell layer. The proposed concept bases on the combination of a self-expanding stent technology with the principles of vascular tissue engineering: The molding process of vascular grafts, based on a fibrin gel scaffold, allows the complete integration of a self-expanding stent structure within the tissue-engineered vessel. With this completely new principle the major causes of restenosis (i) the foreign body reaction, (ii) the cell proliferation with ingrowth in the lumen and (iii) acute thrombosis by hemoincompatibility will be prevented. The reason is the total exclusion of the atherosclerotic section from the blood stream and the coating of the neolumen with a functional endothelium, including the antithrombotic function of the endothelial cells. **Methods:** Small caliber (6 mm) BioStents were made by combining a self-expanding nitinol stent with a thin fibrin-based tissue-engineered blood vessel. The remodeling of the fibrin scaffold with mature autologous proteins was tested by histological analyses. A confluent endothelial cell monolayer lining the luminal surface of the BioStent was shown by scanning electron microscopy. **Results:** A thin coverage of about 200 µm completely wrapping the stent structure was achieved. With the scanning electron microscopy a total lining with endothelial cells could be observed. **Conclusions:** The present feasibility study shows the successful combination of a self-expanding nitinol-stent with a fibrin-based tissue-engineered blood vessel. Further investigations like integrity of the luminal endothelial cell layer and in-vivo studies are necessary to proof a percutaneous applicability and will be implemented soon.

Dialysis Techniques Access—General Session

O132 (EI0021)

From the Dialysis Outcomes and Practice Patterns Study (DOPPS): The Impact of Seasons on Central Venous Catheter Related Septicemia

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Objectives: While central venous catheter related sepsis (CRS) contributes to adverse health outcomes in hemodialysis (HD) patients, unexplored risk factors remain, such as season of the year and central venous catheter (CVC) dressing protocols. **Methods:** Data of 8412 HD patients in 12 countries from DOPPS I and II (1996–2004) were analysed. CRS was defined as septicemia during or within 15 days after HD CVC use. Catheter time at risk (n = 1,754,293 days) and CRS were assigned to 1 of 4 seasons in each country. CRS relative rates (RR) by season and the association of facility CVC dressing protocols with hazard ratio (HR) of CRS were determined by Poisson and Cox regression, respectively. **Results:** Overall CRS rate was 1.2/month or 0.41/(1000 CVC days) (0.47 in North-America), ranging from 0.34 during 'fall' and 'winter', over 0.38 in 'spring', to 0.49/(1000 CVC days) during 'summer'. CRS varied by month with a maximum CRS in August (0.57/(1000 CVC days), and an adjusted RR for "summer" of 1.42 [95% CI (1.09–1.87)] compared to reference 1 for "winter." With respect to the dressing protocol, CRS was lower using betadin [adjusted HR = 0.81, 95% CI (0.65,0.996)], or chlorhexidine [HR = 0.81 (0.59,1.11)] compared to alcohol, with attenuated HRs (0.87–1.06) for combined cleansing agents. Furthermore, CVC infection rates varied by personnel type who typically inspects the CVC access site and/or changes dressing protocol: nephrologist [HR = 0.64 (0.45,0.92)], technician [HR = 1.47, (0.98,2.2)] compared with nurse as reference. **Conclusions:** The higher CRS rate in summer may be due to higher heat, humidity, and perspiration, potentially facilitating bacterial growth and compromising protective measures. Extra vigilance by staff may reduce CRS in this high risk season. betadin and chlorhexidine may be more effective than other cleansing agents.

O133 (EI0024)

Hemodynamic Impact of Helical Designed Grafts: Comparison with Straight Conventional Grafts

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Objectives: In case of low quality vessels, artificial arteriovenous grafts (AVGs) offer the best long-term vascular access for hemodialysis. AVGs, however, deal with complications such as thrombosis and stenosis, the latter finding its origin in intimal hyperplasia which is mainly located in regions of low time average wall shear stress (TAWSS), high Oscillatory Shear Index (OSI) and high relative residence time (RRT), mainly at the venous anastomosis or in the draining vein. The hypothesis that a helically designed AVG reduces intimal hyperplasia formation through the reduction of these unfavorable hemodynamic conditions is studied. **Methods:** Four 3D CFD-models of an AVG (6 mm diameter) between an artery (4 mm diameter) and a vein (6 mm diameter) in loop configuration, were studied: one conventional straight graft design, and three helical designs with a pitch of 105 mm, 70 mm and 35 mm, respectively. All models were meshed with a hexahedral, structured and conformal grid. A physiological pulsatile blood flow was used as inflow (average 600 ml/min), while the distal artery and vein had an outflow of 5%, and the proximal vein 90%. **Results:** The area of TAWSS below 1 Pa lowered by 12%, 0.8% and 60% for progressively more helicity in the design, whereas the area of OSI above 0.1 decreased by 7%, 61% and 80% and the area with an RRT above 1 Pa-1 with 12%, 13% and 55%. All helical designs thus reduced the area of low TAWSS, high OSI and high RRT. The design with pitch 105 mm already lowered the area with high OSI, while only the strong helical design (pitch 35 mm) lowered the areas with low TAWSS and high RRT. **Conclusions:** The areas with unfavorable hemodynamic conditions at the upper wall of the venous anastomosis can be lowered using a strong helical design but not fully eliminated.

O134 (EI0031)

Alternative Dialysis Vascular Access in Comparison to Standard Radial-Cephalic Arteriovenous Fistula

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Objectives: Arteriovenous fistula (AVF) between the radial artery and the cephalic vein at the wrist is the most preferred configuration of dialysis vascular access. However, in patients with inadequate cephalic veins that are not suitable for constructing standard AVFs, we need to create alternative AVFs at different sites or using nonnative materials. In this study, we evaluated the characteristics and AVF survivals in the patients with standard and alternative AVFs. **Methods**: We constructed a standard radial-cephalic AVF at the non-dominant wrist when the cephalic vein was sufficiently large (approximately 2 mm or larger in diameter). Meanwhile, when the vein was not available, we constructed an alternative AVF at a different site or using a prosthesis. In both groups, medical records including age, gender, comorbid conditions and a history of surgical procedures were retrospectively analyzed. In addition, primary and assisted survival of AVFs which were created in the recent one year were also determined. **Results**: In 305 first-time vascular access surgery performed from 2006 to 2010, we constructed 207 standard AVFs (68%) and 98 alternative ones (32%) such as antecubital AVFs. The patients with alternative AVFs, compared with standard ones, were significantly older (71.8 y.o. vs 67.7 y.o.) and more female-gender (39% vs 27%). A history of previous surgery seemed to significantly reduce the likelihood to create standard AVFs (odds ratio 2.55, 95%CI 1.41–4.62, p = 0.002). Although alternative AVFs required more frequent radiological intervention (30%), their assisted one-year survival was 79%, which was as high as that of standard AVFs (94%). **Conclusions**: It seemed that a history of previous surgery was a risk factor for successful standard AVF construction, suggesting that peri-operative venipunctures potentially affected the vascular availability. It also seemed that alternative AVFs, once created at different sites with available veins, are comparably useful as dialysis accesses.

O135 (EI0035)

Suspected Catheter-Related Bloodstream Infection—Nontunneled Vs Tunneled Hemodialysis Catheters

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Objectives: Complication of central venous catheterization for hemodialysis are catheter thrombosis and catheter-related bloodstream infection (CRBI).

We report rates of bacteremia experienced with all types of central venous catheters (CVC). We used femoral and subclavian catheters as non-tunneled CVC (NTCVC) and tunneled CVC (TCVC)- femoral, subclavian and jugular catheters. **Methods:** In a prospective study we looked at the outcome of a group of 620 patients (pts) with acute and chronic renal failure treating with hemodialysis via a 738 CVC,during the 3-year period. Catheters were placed by nephrologists in a femoral, internal jugular and subclavian vein, and episodes of catheter infection were recorded. Each catheter was followed individually until it was removed or until the end of the study. **Results:** In total, 539/738 (74.9%) of procedures were insertions of NTCVC, whilst 181/738 (25.1%), were insertions of TCVC. Of the NTCVC insertion, 501/738 (69.6%) were inserted into femoral vein, with 38/738 (5.3%) in subclavian vein. In a group of TCVC—103 were tunneled femoral (56.9%), 41 tunneled jugular (22.6%), and 37 subclavian (20.5%) catheters. A total of 44 576 catheter days were accumulated over the study period during wich time were 42 cases of CRBI (infective rate 2.57 episodes/1000 catheter days). Rates of CRBI were 2.77 episodes/1000 cath. days in the TCVC group; 3.5 per 1000 cath days in NTCVC. Multivariant analysis demonstrated hazard ratios (HR) for the development of CRBI in pts dialysing with subclavian NTCVCs of 2.9 [95% confidence interval (CI) 1.5–4.8, $p < 0.001$], and femoral NTCVC of 3.9 [95% CI 2.1–7.4, $p < 0.001$]. The infection and malfunction free survival time was with statistically significant difference between NTCVC and TCVC. **Conclusions:** We concluded that recognizing and knowing the risk factors can prevent complications of tunneled hemodialysis catheters. TCVC insertions have an association with lower complication rates than NTCVC insertions

O136 (EI0056)

A New Method of Monitoring Blood Pressure and Cardiac Output in Chronic Dialysis Patients

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Objectives: Hypotension and circulatory failure are common problems during dialysis sessions. These problems occur often suddenly without any early signs. Short-term noninvasive blood pressure measurement (method of Riva-Rocci) is often not tolerated by the patient so that a decreasing blood pressure is not detectable early enough. A new circulation monitoring system would be helpful. **Methods:** We established a method for the measurement of the blood pressure in the arteriovenous shunt of chronic dialysis patients during discontinued blood flow in the vascular access. A measurement and analysis system evaluates the pressure fistula values, which correlate with systemic blood pressure and analyse further parameters (slope of the increasing mean arterial pressure after stopping the blood flow in the vascular access,waveform of every single pulse). We investigated 10 stable chronic dialysis patients, suffering from chronic heart failure and hypotension during dialysis sessions. The systemic blood pressure was measured with an automated device (Dinamap, GE Helthcare). Measured values of both systems were compared every 15 minutes. **Results:** Measured values of both methods differed, but the trend during a dialysis session was the same. The blood pressure in the shunt and especially the slope of the blood pressure after stopping the blood flow in the vascular access depend on shunt characteristics (age, location, maximum blood flow, prevalence of stenosis). The maximum blood flow in the shunt depends on systemic circulation. The new measurement procedure is well tolerated by the patient, altough it is performed in a very short term. The sensitivity of the detection of decreasing blood pressure values is improved. Early signs of circulation failure are detectable, especially if the evaluation of fistula blood flow characteristics is obtained. **Conclusion:** The new measurement is a save method for detecting circulatory failure during dialysis sessions in patients with high risk of circulation failure.

O137 (EI0207)

Two Single-Lumen Non-Cuffed Catheters in the Jugular Vein as a Hemodialysis Vascular Access for More Than 100 Days

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Objectives: Two single lumen catheters in the same jugular vein have been used as a preferred vascular access in our hemodialysis (HD) patients since 2006. With this study we wanted to retrospectively analyze clinical outcome of such a vascular access and reasons for catheter removal in those patients where catheters were not removed for at least 100 days. **Methods**: In 121 adult patients (53 females and 68 males) as a vascular access two single lumen precurved 8 F catheters (Medcomp, Harleysville, PA, USA) inserted in the same jugular vein were used between January 2009 and April 2010. 30 % solution of a 3Na-citrate was used as a locking solution, and 2 % Mupirocin ointment was applied on the exit site routinely. Analysis was performed 6 months after the end of inclusion period. **Results**: In 20 patients (10 females and 10 males, age 70.2 ± 11.3 years) catheters were not removed or exchanged for at least 100 days. At the time of analysis in 4 patients catheters were still functional. 3 patients died with functional catheters. The longest duration of such a vascular access was 387 days and this patient died with the functioning and not infected vascular access. In 6 patients catheters were removed because AV fistulas were constructed. In 6 patients exchanges over guide wires were performed for correction of displaced functional catheters, and in 1 patient catheters were, because of an infection, removed at day 184. **Conclusions**: Two noncuffed single lumen precurved 8 F catheters inserted in the same jugular vein, locked with 30 % citrate, seem to be a safe long lasting vascular access for some HD patients, but further prospective studies are needed to evaluate clinical outcome and complications of such a vascular access.

Scaffolds for TE via Electrospinning-Structures and Biomaterials—Symposium

K21 (EI0445)

Designing Electrospun Scaffolds for Tissue Engineering

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Objectives: Electrospinning is a facile technique for production of polymer fibres with diameters in sub-micron to micron range. Resulting scaffold microstructure closely resembles the extracellular matrix and can be enhanced further by incorporating various signalling molecules such as proteins, growth factors etc. in fibres. This is achieved via modifications to the standard electrospinning process *via* blend, emulsion and co-axial electrospinning. **Materials and Methods:** Water-soluble polymers such as PEO were functionalised by blend electrospinning by mixing the protein solutions with polymer solution prior to electrospinning. Stability of the fibres was achieved through a crosslinking step postelectrospinning. Water-insoluble polymers were functionalised using emulsion and co-axial electrospinning. For the former, protein solutions were emulsified in water-immiscible polymer solutions with a surfactant, while for the latter, a specially designed concentric nozzle setup was employed. Fibres were collected on different collector geometries to obtain different scaffold macrostructures. **Results:** The process was influenced by different parameters such as concentration, voltage, flow rate, etc. Proteins were incorporated in fibres and released via diffusion. For blend electrospinning, burst release of proteins was observed along with protein denaturation. Both problems were solved by using the modified electrospinning procedures (emulsion or co-axial electrospinning). Different 3D constructs such as heart valve conduits and tubes for vascular grafts were produced. **Conclusions:** The electrospinning process could be easily modified to allow functionalisation of fibres with proteins. Of the 3 different methods, blend electrospinning was the least favourable, since it caused protein denaturation. On the other hand, protein activity was retained using emulsion or co-axial electrospinning for fibre functionalisation. 3D macrostructure heart valves or blood vessels could be replicated by using different collector geometries. This work is supported by funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for the Cluster of Excellence REBIRTH (From Regenerative Biology to Reconstructive Therapy).

O138 (EI0063)

Membrane Bioengineering Strategies Towards The Development of a Biological Kidney Support Device

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Objectives: Despite hemodialysis therapy, many end-stage renal disease patients have uremic complications, resulting in a poor quality of life. Uremia is caused by the retention of a large group of molecules that are not adequately cleared by hemodialysis. Attempts to improve this situation by convection or high-flux dialysis membranes were not very successful. In our study, we develop living membranes for removal of protein bound uremic toxins by renal epithelial cells. In our concept, we use a composite membrane having two layers: the first layer allows protein bound toxins to reach the cells but simultaneously blocks immune cells, immunoglobulins and complement factors. The second layer made of bioactive electrospun mesh favors the adhesion of renal epithelial cells which will assist on the toxins removal. **Methods:** For the first layer, Flat and hollow fiber (HF) membranes were either prepared in house by phase inversion or purchased For the second layer, polycaprolactone (PCL) based fiber mesh was produced by electrospinning and integrated with the first membrane layer. The morphology of the produced membranes was investigated via SEM, whereas the transport properties were studied using water and protein model solutions at selected pressures. **Results:** For the protein permeable membrane, polysulfone based homemade or commercial membranes were studied. The tested membranes have high water permeance (1500–2200 L/hr/m^2/bar), high albumin sieving coefficient (SC, higher than 0.8) and rather low immunoglobulin IgG transport (SC < 0.3). The cytocompatible fiber mesh was obtained by electrospinning of polycaprolactone (PCL) and integrated onto the protein permeable membrane by either direct electrospinning or lamination. **Conclusions:** Direct electrospinning compromises water and BSA transport through the composite whereas the transport remained unaffected when the PCL membrane is produced separately and later laminated onto the mem-

brane. Besides flat composites, the preparation and characterization of capillaries is in progress, too.

O139 (EI0220)

Assessment of Aliphatic Thermoplastic Polyurethanes for Narrow Diameter Vascular Grafts

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Objectives: Thermoplastic polyurethanes (TPUs) find use in demanding medical applications such as catheters and pace maker casings. In addition to good resistance to thrombosis, TPUs have better mechanical compliance than traditional vascular graft materials (Dacron or PTFE) and are actively researched for use as small bore (<5 mm) grafts. Most commercially available TPUs are based on aromatic isocyanates which offer excellent mechanical properties and stability however raise concerns over toxicity of decomposition products. Such concerns are compounded if the graft is intended to be fully or partially degradable; two approaches in vascular grafting which seek to assist rather than replace native tissue growth. **Methods:** TPUs based on aliphatic isocyanates, oligomeric diols and different ester containing chain extenders have been synthesized. Polymers were fabricated into forms for mechanical testing and simulated biodegradation. Toxicity was assessed both in vitro with HUVECs and in vivo by subcutaneous implantation. Aliphatic TPUs were also electrospun into nanofibrous conduits and implanted as aortic interponates into Sprague rats. **Results:** Mechanically, aliphatic TPUs tend to be softer than aromatic TPUs although modulus can be adjusted with appropriate chain extenders. Procedures for electrospinning tubes from TPUs are also amenable to aliphatic derivatives. Fiber diameter, mesh density, and wall thickness are all well controlled. HUVECs seeded on aliphatic TPUs attach and grow well. Live/ dead assays maintain that cells stay viable in the presence of polymer and its degradation products. Initial results from implantation show no complications. **Conclusions:** Aliphatic TPUs with cleavable chain extenders have flexible mechanical properties and provide potential advantages in degradable or partially degradable vascular grafts. Tuning of modulus and rate of degradation is possible by blending or copolymerization. Electrospinning TPUs provides nanofibrous tubes appropriate for vascular grafting.

O140 (EI0064)

Integration of Hollow Fiber Membranes Improves Nutrient Supply In 3D Tissue Constructs

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Objectives: Despite the great progress in tissue engineering, development of clinically relevant size tissues with complex architecture remains a great challenge mostly due to limitations of nutrient and oxygen delivery to the cells. This study focuses on the development and utilization of a new perfusion culture system using hollow fiber membranes to provide adequate nutrient delivery to the cells within large three-dimensional (3D) scaffolds. **Methods:** Three dimensional scaffolds were created by (i) rolling preseeded electrospun sheets around porous hollow fiber (HF) membranes and (ii) by integration of HF within free form fabricated (FFF) scaffolds. The culture of pre-myoblast (C2C12) cells under static and dynamic dynamic conditions on these scaffolds was investigated in a dedicated bioreactor. In fact, dynamic medium perfusion occurred via the HF lumen and around the 3D scaffolds. Various parameters such as fiber transport properties, fiber positioning within a scaffold, and medium flow conditions were optimized. The scaffolds were analyzed using scanning electron microscopy (SEM), histology and DNA assay. **Results:** The hollow fibers act as additional source of nutrients and oxygen to the cells by providing medium through the porous walls in a controlled manner at low shear stress. The SEM analysis and histology shows clearly that only integration of fibers achieves homogenous cell distribution within the scaffolds whereas the total DNA assay shows quantitatively high cell proliferation within the scaffolds. In the case of the electrospun multilayer scaffolds, cell migration occurs within the construct (shown using prelabeled C2C12 cells) illustrating the potential of using our concept for developing more complex tissues. **Conclusions:** This study demonstrated the proof of concept of using polymeric hollow fibers as artificial capillaries for nutrient delivery to rather large 3D tissue constructs and could provide a basis for a new culture methodology for developing such constructs.

O141 (EI0355)

Electrospun Fish Gelatin: Effect of Cross-Linking Methods on Cell Growth

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Objectives: This study was performed in order to produce electrospun fish gelatin scaffolds and evaluate the effect of different cross-linking methods on cell growth and proliferation. **Methods:** Fish gelatin (from fish skin—Sigma G7041) is soluble in cold water. In this work, it was dissolved in: distilled water, at a concentration of 40 wt% (solution S1); acetic acid:distilled water (90:10 in wt) at concentrations of 25 wt% (S2) and 18 wt% (S3). The cross-linking methods used were: electrospun mats obtained from S1 and S2 were exposed to glutaraldehyde vapor or dehydratated at low pressure and high temperature; 2% genipin was added to S3 and the solution was electrospun after 6 days. The mats obtained from S3 were then immersed in an ethanol:water (90:10 in wt) solution with 2% genipin in order to increase the cross-linking degree. Scanning electron microscopy was used to characterize the morphology of the scaffolds and effectiveness of the cross-linking procedure. 3T3 fibroblasts were cultured on the fiber mats and confocal microscopy images were used to observe the morphology of the cells by immunostaining with phaloidin and ToPro3. **Results:** Electrospun gelatin nanofibers obtained from S1 and S2 were defect free with regular fibre diameters while those from S3 presented varying diameters and some fiber bonding. After cross-linking, some structural degradation occurs, in particular with glutaraldehyde and genipin. All nanofibrous mats became insoluble after cross-linking. Confocal images revealed good adhesion and proliferation of the cells cultured on all scaffolds, with those cross-linked with genipin showing the best results. **Conclusions:** Solutions of fish gelatin were successfully electrospun. The scaffolds were cross-linked using three different methods. The cell culture study showed that cross-linked gelatin from fish skin is a promising scaffold for soft tissue engineering.

Drug Delivery Systems—General Session

O142 (EI0155)

Tat Protein Transduction Into Isolated Perfused Heart and in Vivo During Cardiopulmonary Bypass

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Objectives: Linkage of the 11-amino-acid transduction domain of HIV TAT to a heterologous protein allow the protein to be transduced readily across cellular membranes into cells. The aim of the study was to investigate the effectiveness of delivery of peptides into cardiomyocites *ex vivo* in isolated perfused heart and *in vivo* during cardiopulmonary bypass (CPB) using the newly developed TAT protein transduction system. **Methods**: TAT undecapeptide fused with green florescent protein (TAT-GFP) was infused *ex vivo* in isolated perfused heart (Langendorff) model for 60 minutes. Secondarily TAT-GFP was infused *in vivo* in a rat model of CPB that last for 60 minutes. These procedures were followed by a 15-minute washout period, which was followed by perfusion-fixation with formaline. **Results:** Confocal microscopy revealed homogeneous distribution of TAT-GFB in myocardium sliced from apex to base of the heart. TAT-GFP was localised in >90% of cardiomyocytes both *ex vivo* and *in vivo*. **Conclusions**: These results demonstrate that TAT protein transduction may be a promising tool for myocardial protection in cardiac surgery.

O143 (EI0264)

Growth Factor-Delivering Devices for Tissue Engineering Applications

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Objective: The rationale for the use of growth factors in tissue engineering applications is given by their ability to stimulate the controlled proliferation and differentiation of the seeded cells in the scaffolds. They should also enhance the migration, proliferation, and differentiation of cells from the edges of the treated defect. We analysed three different approaches for integration of a potential growth factor prostaglandin E_2 (PGE_2). **Methods:** The first approach utilizes an emulsion-based route to synthesize polymeric (PLGA) microspheres with incorporated PGE_2. These microspheres were used in a second approach to establish a three-dimensional scaffold system by distributing PGE_2-PLGA-microspheres in a gelatinous suspension followed by freeze-drying and moderate chemical cross-linking. The third system used the development of a PLGA scaffold with direct integration of the biosignal. All PGE_2 values were given as mean values after triple analyses via mass spectrometry assays. **Results:** The first approach showed a release kinetic of biologically

active PGE_2 with a burst release of PGE_2 over the first two days and continued release over 8 days. The distribution of growth factor-loaded microspheres allows a gradient of growth factors due to the spatial distribution of the microspheres. The incorporation efficacy of the fluid-foamed system was higher than different preparations of microspheres. This third approach demonstrated that the direct incorporation of PGE_2 into a polymer foam is possible without extensive loss of the growth factor. **Conclusions:** Growth factors act in a dose-dependent manner and via receptors on the target cells. Therefore, the kinetics of growth factor release from delivering devices should be adapted to the situation of the microenvironment. The amount of growth factor in the tissue or the cell-scaffold construct must reach an optimum for its biological action in balance which is one major feature for the biomaterial design.

O144 (EI0175)

Amphiphilic Block Copolymers: Synthesis and Characterization of Multifunctional Micelles for Targeted Drug Delivery by Raft Polymerization

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Objectives: Reversible addition-fragmentation chain transfer (RAFT) polymerization belongs to the controlled/living radical polymerization methods and is highly efficient in preparation of well-defined drug and gene delivery vesicles. **Methods:** Via RAFT Polymerization different amphiphilic block copolymers consisting of poly(N-acryloyl morpholine) or poly(N-isopropylacrylamide) and poly(2-hydroxyethyl methacrylate-block-poly(ε-caprolactone)) have been prepared. Two different trithiocarbonate RAFT agents have been used and AIBN acted as initiator. As fluorescence marker in cell imaging Fluorescein methacrylate was copolymerized in the hydrophobic block. Micelles were formed by dialysis and their hydrodynamic diameter was characterized by dynamic light scattering (DLS). **Results:** We successfully prepared well-defined amphiphilic block copolymers with low polydispersity indices by RAFT polymerization. They composed of poly(N-acryloyl morpholine) and poly(N-isopropylacrylamide) as hydrophilic part and biodegradable poly(2-hydroxyethyl methacrylate-poly(ε-caprolactone)) in the hydrophobic part. The ability of these block copolymers to self-assemble into micelles in an aqueous surrounding was determined by DLS. The uptake of the micelles into living cells was shown by fluorescence imaging. Further modification of the block coplolymers is possible due to succinimide units in the polymer backbone. **Conclusions:** Based on biodegradable amphiphilic block copolymers we designed new smart drug carriers which can assemble into micelles. Because of succinimide groups in the hydrophobic part these block copolymers are able to bind drugs with an amino functionality. The fluoresceine moiety included in the polymer backbone allows tomonitor the cellular drug uptake by fluorescence imaging. Enzymatic degradation studies of the micelles are currently ongoing.

O145 (EI0044)

Dual Pro-Angiogenic Therapy Based on The Release of Vegf165 and Endothelial Cells Derived From Umbilical Cord Stem Cells In A Hybrid Network

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Objectives: We report a new approach to integrate in the same 3D hydrogel scaffold a growth factor delivery system and a matrix able to support the adhesion and remodeling of endothelial cells derived from hematopoietic stem cells. **Methods:** Dextran with variable degree of oxidation (DexOx) was conjugated with $VEGF_{165}$ and the conjugates characterized by SDS-PAGE and circular dichroism. The bioactivity of the conjugates was evaluated in $CD34^+$-derived endothelial cells (ECs) by their potential to trigger Ca^{2+} uptake and to activate ERK and Akt signaling pathways. Radiolabelling and ELISA were used to monitor VEGF release from dexOx-VEGF hydrogels. DexOx-VEGF microgels were encapsulated in fibrin hydrogels, loaded with ECs, and the cellular viability was evaluated up to 6 days. During this period, the organization and gene expression of ECs was evaluated by microscopy and qRT-PCR. **Results:** VEGF was immobilized into DexOx having variable degree of oxidation (DO). Circular dichroism results show preservation of the 3D structure of immobilized VEGF. In addition, immobilized VEGF activates the intracellular concentration of Ca^{2+}, ERK and Akt signaling pathways. Dextran hydrogels containing immobilized VEGF have variable release rates depending on their crosslinking density. The release of VEGF within the hybrid gel induced a cord-like organization of the endothelial cells. This effect was only observed for constructs containing low oxidized dextran hydrogels. In addition, no visible network formation was observed in low DO constructs without VEGF, indicating that the formation of cord-like structures was mediated by VEGF. Finally, constructs that release faster VEGF present cells with a downregulation in the expression of VEGF gene and an upregulation of MMP-2 gene. **Conclusions:** The hybrid construct allows the independent tailoring of the drug release system from the cell-bearing matrix and was shown to modulate gene expression and three-dimensional endothelial cell organization.

O146 (EI0033)

Effect of Liposome-Encapsulated Hemoglobin After Transient Cochlear Ischemia and Reperfusion in The Gerbil

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Objectives: Liposome-encapsulated hemoglobin (LEH), an artificial oxygen carrier, has been proved to be protective when administered before cochlear ischemia and reperfusion. In the current study, LEH was tested after transient cochlear ischemia and reperfusion as a therapeutic model of sudden deafness. **Methods:** Mongolian gerbils, devoid of circle of Willis, were randomly assigned to receive 2 ml/kg of LEH with high O_2 affinity ($P_{50}O_2$ = 17 mm Hg) or saline (each n = 6) one hour after 15-min occlusion of the bilateral vertebral arteries to induce transient cochlear ischemia. Sequential changes in hearing were assessed by auditory brain response at 1, 4, and 7 days after ischemia/reperfusion, when the animals were sacrificed for pathological studies. **Results:** LEH was significantly more effective than saline in suppressing hearing loss over a wide auditory range at 8 kHz, 16 kHz, and 32 kHz in contrast to the saline treatment which was associated with significant hearing loss in each auditory range ($P < 0.05$). Although the degrees and differences in hearing loss were gradually reduced as time passed after ischemia/reperfusion 1, 4, and 7 days, when the inner hair cell loss was significantly suppressed in LEH-treated animals ($P < 0.05$). **Conclusions:** The results suggest that postischemic treatment with LEH (2 ml/kg) with high O_2 affinity ($P_{50}O_2$ = 17 mm Hg) is effective in mitigating hearing loss (function) and inner hair cell loss (morphology) following transient cochlear ischemia and reperfusion as an experimental model of sudden deafness.

O147 (EI0253)

Morphology of Polymer Matrices Containing Risperidone

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Objectives: Nowadays innovation in the therapy of schizophrenia is achieved by applying novel atypical antipsychotic agents and also by novel drug formulations. Long-acting risperidone in the form of injection is a relatively recent solution acting during two weeks. Acting agent in commercial product is microencapsulated in poly(lactide-co-glycolide) and formed in microspheres. We propose implantable and biodegradable solid form that allows prolonged action and explantation in comparison with commercial product. **Methods:** Risperidone loaded matrices (10 wt-% of drug substance) were obtained from high molecular poly(L-lactide-co-glycolide) 85:15 by solution casting method. Morphological properties of polymeric matrices were evaluated by atomic force microscopy (AFM) during 9 months of degradation. Degradation processes of polymeric matrices with diameter of 10 mm was carried out in phosphate buffered saline (pH 7.4) at the temperature of 37°C under the constant agitation conditions. **Results:** During degradation processes the changes in morphological and morphometric features between nondegraded and degraded matrices were revealed. Nondegraded matrix showed solid structure with single pores. Incubation of matrices in phosphate buffered saline under the constant agitation conditions resulted in the appearance of significant porosity. Moreover, elongation of degradation period influenced the widening of pores' diameter and their deformation. **Conclusions:** AFM study showed differences between risperidone-loaded matrix before and after 9 months of degradation, thus confirmed progression of degradation processes. Determination of morphological and morphometric changes during degradation process are important for designing biodegradable and implantable matrices releasing risperidone.

Approval Procedures for Medical Devices: Facts, Figures and Basic Rules Seen From Different Continental Perspectives—Artificial Organs and Society: Recent Trends in Japan

O148 (EI0277)

Current Status of Industry-Academia Collaboration Activities in Japan and Practice of "National Cerebral & Cardiovascular Center"

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Objective: To develop new medical devices such as ventricular assist devices which will be used in clinical, it can be considered that industry-academia collaboration had better to be conducted to lead by medical research institute. Medical research institute in Japan had not generally conducted industry-academia collaboration activities organizationally, whereas industry-academia collaborations have become active in accompanied by shifted to independent administrative agency in the universities. The objective of this study is to make clarify the current status of industry-academia collaboration in Japan including National cerebral & CardioVascular Center (NCVC), owing to promote technology commercialization on medical device development. **Methods:** The present activities of industry-academia collaboration of NCVC was compared with that of universities which surveyed by the Center of the Ministry of Education, Culture, Sports, Science and Technology Japan. To compare the activities of industry-academia collaboration, the number of joint research contracts and patent royalty income were selected as indicators. **Results and Conclusion:** The number of joint research contracts of universities in Japan during last year (within the top 10) was about 300–1000 and patent royalty income was 340–1 100 thousand U.S. dollars. On the other hand, department of intellectual asset management was established with the independent administrative agency shift of NCVC on April 2010, and promoted industry-academia collaboration activities. Under this new organization, NCVC entered into 52 joint research contracts and patent royalty income was 70 thousand U.S. dollars during last year. NCVC has only about 200 researchers and medical doctors, whereas these universities have about 2 000–4 000 faculty and researchers, and hence these universities hold about 10–20 times manpower of NCVC. Considering manpower, our performance of joint research and patent royalty income is equal to within the top 10 universities. These suggest that NCVC can be propelled industry-academia collaboration effectively.

O149 (EI0146)

Wide Acceptance for Domestic Medical Devices in Japan

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Objective: As for medical device business, harmful rumors often cause withdrawals of existing companies or entry barriers for new companies. Many Japanese manufacturing companies, especially small and medium enterprises, hesitate to enter into medical device business due to the fear for harmful rumors although they can provide high quality technologies. The objective of this study is to investigate the public attitudes toward the medical device field in Japan. **Methods:** We designed an internet-based questionnaire providing basic information on current status of the medical device field and assessing the public opinion on the issue; necessity, self-sufficient rate, approval, review periods, recalls, information, corporate images of the companies entering into medical device business from other business fields and attitudes toward Japanese medical device companies. The survey included a nationally representative sample of 5,155 adults excluding health care workers. The study period was from March 8, 2011 to March 9, 2011. **Results:** Among the respondents, 94% recognized the growing necessity of medical devices; 83% required self-sufficient rate improvement of medical devices. As for regulation and safety, 27% poorly understood that medical devices were approved by a government organization; 63% expected shorter review periods; 90% realized that recalls contributed to the safety. Of the respondents, 80% expected to be provided technical information. Concerning medical device companies, 63% considered that corporate image of the companies which entered into medical device business from other fields would improve; 99% supported for the domestic medical device companies. **Conclusions:** The study revealed the wide acceptance for domestic medical devices in Japan. Japanese highly recommended contributions of domestic companies to medical device development. The public attitudes may motivate Japanese manufacturing companies to enter into medical device business.

O150 (EI0364)

Artificial Organs in Complex Emergencies of Earthquake, Tsunami, and Nuclear Power Plant Accident

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Objectives: Magnitude 9.0 earthquake and over 30 meter tsunami attacked the Tohoku area in March 11, 2011. Furthermore, steam explosion attacked Fukushima nuclear power plant and radiation exposure was observed in Tohoku area. Details concerning artificial organs in Tohoku area will be presented in this session. **Methods:** All system in hospitals in Miyagi prefecture downed. Dialysis patients gathered to the Tohoku University Hospital, which have first priority in life line in Sendai city. HD patients transported another national hospitals by the helicopter. Monitor, respirator and ventricular support system were smoothly recovered after electric power supply. **Results:** Dead or alive was decided in border of the tsunami area. Patients with artificial orgams were almost OK in Tohoku University Hpspital. Water supply was damaged in city level, so it became the disturbance of HD. A lot of HD patients was transported by helicopter. Number of Helicopter were too small in Sendai. **Conclusions:** Repeated discussion must be performed for the emergency situation of the the artificial organs.

Artificial Organs

P1 (EI0184)

Accumulation Rate of Ages in The Skin Biopsy Tissue of Dialysis Patients

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Objectives: To measure the accumulation of different advanced glycation end products (AGEs) in period of 1 year in diabetic hemodialysis (HD) patients and to find the factors that influence their accumulation. **Methods:** Twenty diabetic HD patients were enrolled in this study. Skin biopsy was performed twice with an interval of one year. High performance liquid chromatography was performed on the skin biopsies in order to measure their pentosidine, carboxymethyl-lysine (CML) and carboxyethyl-lysine (CEL) content. Skin autofluorescence (AF) was used as an additional method to estimate the AGEs accumulation. Dietary records from the HD patients were obtained to assess the calorie, protein and AGE intake. Body Mass Index (BMI), as a measure of nutritional state, was calculated. **Results:** Pentosidine (59.9 ± 43.6 vs. 83.5 ± 59.9; p = 0.002), CML (1529 ± 1038 vs. 2050 ± 1204; p = 0.012) and CEL (505 ± 387 vs. 715 ± 658 ;p = 0.015) were significantly increased in skin biopsy specimens during the period of one year. Skin AF correlated with pentosidine, CML and CEL from the skin biopsies (R = 0.902 p = 0.001; R = 0.875 p = 0.001; R = 0.654 p = 0.002). Pentosidine, CML and CEL content of the skin biopsies did not correlate with the calorie, protein and AGEs intake. In the multivariate analysis we found that independent predictors of the annual increase of pentosidine were: CRP (p = 0.038), the annual increase of Skin AF (p = 0.01) and BMI (p = 0.041). The annual increase of Skin AF (p = 0.021) and BMI (p = 0.010) were also the independent predictors of the annual change of CML, whereas the independent predictor of the annual change of CEL was the annual increase of Skin AF (p = 0.001). **Conclusions:** Skin AGEs increased rapidly in diabetic HD patients. The Skin AF is a good estimate of the AGEs accumulation. The BMI and CRP are independent predictors of AGEs accumulation.

P2 (EI0340)

Nephrolithiasis in Autosomal Dominant Polycystic Kidney Disease

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Objectives: The prevalence of nephrolithiasis is considerably greater in patients with autosomal dominant polycystic kidney disease, than in general population. The anatomic factors because of increased intrarenal obstruction, such as cyst growth, renal tubular stasis and methabolic disorders, are important and may predisposed to stone formation. **Methods:** In order to evaluate the nephrolithiasis in polycystic kidneys, 60 patients with autosomal dominant polycystic kidney disease, mean age 42.6+/– 12.8 years, underwent echosonography and computed tomography scan. Routine blood analysis and urine samples, including 24 h urine collections were done. **Results:** Renal stones were detected in 22 out of 60 patients (36,6%). The morphologic data presented that patients with autosomal dominant polycystic kideny disease and nephrolithiasis had more renal cysts and larger predominant cyst size than patients

without nephrolithiasis ($p < 0,05$). Renal function expressed by creatinin clearance was also different between the 2 groups of patients (72,6+/–9,4 in patients with nephrolithiasis, and 93,7+/–8,6 in patients without nephrolithiasis). Twenty-four hours urine analysis showed that patients with nephrolithiasis had significantly lower urine volumes and levels of uric acid. Three patients had urinary tract obstruction, ureterolithiasis with hydronephrosis, with diminished creatinin clearance, but after desobstruction and elimination of the calculi, the renal function was improved. **Conclusions:** The authors consider that nephrolithiasis is important factor for the progression of the renal demage in patients with autosomal dominant polycystic kidney disease, because of complications that may accelerate the progression of the renal disease and the chronic renal failure.

P3 (EI0312)

Use of A Renal Tubule Cell Line (Hk-2) To Study The Nephrotoxic Potential of Dialysate Taken From High Cut-Off Hemodialysis Treatments In Patients With Light Chain–Induced Myeloma Kidney

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Objectives: Acute kidney injury is common in patients with multiple myeloma (MM), most frequently caused by cast nephropathy a direct consequence of the high serum free light chain (FLC) concentrations present in these patients. In this condition FLC induced cell stress responses are frequently seen in proximal tubular cells (PTCs), which results in production of cytokines and tubulo-interstitial inflammation. Removal of circulating FLC with extended high cut-off hemodialysis (HCO-HD) has recently been studied and rapid reduction in serum FLC levels was associated with improved likelihood of kidney function recovery. The aim of this investigation was to study renal epithelial cell toxicity of dialysate obtained from HCO-HD treatments of MM patients. **Methods:** Spent dialysate collected from HCO-HD treatment sessions of 13 patients with FLC induced AKI were concentrated by filtration with a 5 kDa cut-off membrane. The concentrates were exposed to HK-2 cells, a proximal tubule epithelial cell line from human kidney, for up to 48 hours. The effects on cell morphology and activation were studied by microscopy and by measurement of cytokines in the supernatant using enzyme-linked immunosorbent assay. FLC concentrations were determined by nephelometry, using a particle-enhanced immunoassay. **Results:** Incubation of the FLC containing concentrates (25 µmol and 50 µmol) with HK-2 cells induced the release of interleukins IL-6, IL-8 and monocyte chemoattractant protein-1 (MCP-1) and lead to morphological alterations of the tubular cells. There was a considerable variability among the dialysates obtained from different patients. The amount of FLC that stimulated expression of inflammatory cytokines in the PTCs was well within levels that are seen in patients with MM. **Conclusion:** FLCs removed from the circulation of MM patients by HCO-HD induce pro-inflammatory responses in proximal tubule epithelial cells. These responses represent an important mechanism of the tubulo-interstitial inflammation frequently seen in the kidneys of MM patients.

P4 (EI0247)

Use of Rifle Classification in Patients with Community Acquired Acute Kidney Injury (Ca-Aki)

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Objectives: Acute kidney injury (AKI) is a syndrome with an uncertain follow up and often with a fatal outcome. The RIFLE and AKIN initiatives have provided a unifying definition for AKI. Present study aims at validating most recent AKI classification system in CA-AKI. **Methods:** We analysed the clinical outcome in 112 pts with CA-AKI. We excluded dialysis pts, those with malignancy and with preexisting chronic kidney disease (CKD) or kidney transplantation. RIFLE criteria were implied on admission, with retrospective analysis of previously prospectively collected data. **Results:** Pts median age was 45.5 y, 61.6% were male. 35.7% had 1, 25.4% had 2 and 7.1% had 4 comorbid diseases. Mortality rate was 22,7% and initial mean APACHE2 score was 17.3 ± 7.4. 7 risk factors were implicated in pts outcome. According to RIFLE, pts were classified in stage 1 (Risk) in 1 (0.9%), stage 2 (Injury) in 4 (3.6%) and stage 3 (Failure) in 76 (67.9%). Mortality rate in stage 3 was 18 (16.82%). After 4 w of treatment, we found that 31 (27.7%) where in stage 4 (Lost) with a mortality rate of 7 (6.5%). Univariate analysis of four RF like creatinine(s), age (years), UO and APACHE2 in stage 3 and 4 of RIFLE, in correlation with mortality, were significant only with UO. Pts who died (18 in stage 3 and 7 in stage 4), had lower baseline levels of Creat(s) ($p = 0.028$) and UO (0.017) than those alive at 4 w. Higher APACHE2 score was associated with higher mortality. Kaplan-Meyer surviving courve showed that RIFLE stage 3 pts were with longer surviving in period of 4 w compared to those in stage 4. **Conclusions:** The study supports use of RIFLE as an optimal classification system to stage CA-AKI severity, still there is perhaps a need for use of other new parameters in this type of AKI.

P5 (EI0246)

Renal Affection in Patient with Late Diagnosed Sjögren's Syndrome

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Objectives: Sjögren's syndrome (SS) is the second most common autoimmune disease affecting mainly women. The true prevalence of SS is unknown but is estimated to affect 1–3% of the adult female population. Both tubular and glomerular damage have been described in SS, although glomerular disease is rare. The aim of this report is to present a case of interstitial nephritis with proteinuria in late diagnosed primary Sjogren's syndrome (pSS), aiming to suggest recommendations for treatment. **Methods:** We describe a rare case of primary SS (pSS) in a 76-year-old woman presenting with hypokalemic cardiac arrhythmia, chronic renal failure due to severe tubular and glomerular affection. **Results:** The patient had been diagnosed as having pSS on the basis of dry eyes, dry mouth, weight loss, arthralgia, parotid gland tumefaction, positive SSA and positive Schrimer's test. Clinical presentation at admission was cardiac arrhythmia with acute over chronic renal failure with intermediate range of proteinuria. Patient had more than ten years a hypertension as a medical comorbidity. We performed renal biopsy and found global glomerulosclerosis, with mild tubule interstitial nephritis accompanied with interstitial fibrosis and atherosclerotic changes. Imunohystochemical tissue analysis showed multifocal lymphocytic infiltrate. MDRD at point of renal biopsy was 5.01 ml/min. A treatment with corticosteroids (1 mg/kg/day) was started. Patient was set to chronic dialysis program. Few weeks later she broke her hip and femur fracture was confirmed. Bone mineral density revealed osteopenia of the hip and normal density of spine. Vitamin D levels were low, supporting the diagnosis of osteomalacia. **Conclusions:** The kidney may be a target of the disease in pSS. Although, overt renal disease is rare, latent involvement has been reported in up to one-third of patients. Further studies and successful cases are required to determine indications for and dosages of immunosuppressive treatment in patients with renal involvement of pSS.

P6 (EI0022)

Egfr is A Poor Predictor of Uremic Toxin Concentrations

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Objectives: The degree of chronic kidney disease (CKD) is currently expressed in terms of glomerular filtration rate (GFR), which can be determined directly or estimated according to different formulae based on serum creatinine (SCrea) and/or cystatin C measurements (eGFR). We aimed to investigate whether eGFR-values are representative for uremic toxin concentrations in patients with different degrees of CKD. **Methods:** Associations between eGFR based on serum cystatin C (Stevens) and different uremic solutes (MW range 113–240Da) [SCrea, uric acid (UA), symmetric dimethylarginine (SDMA), asymmetric dimethylarginine (ADMA), and free and total hippuric acid (HA), 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), indoxyl sulfate (IS), indole acetic acid (IAA), and p-cresylsulfate (PCS)] were evaluated in 95 CKD patients not on dialysis. The same analysis was applied for 6 other eGFR formulae. **Results:** There was a substantial disparity in fits among solutes. In linear regression, SCrea showed the best model fit (Stevens $R^2 = 0.605$), while explained variance of eGFR was extremely low for the majority of solutes with R^2 in the range 0.4–0.2 for total IS, SDMA, free IS, and free IAA, and even below 0.2 for ADMA, free HA, free pCS, total HA, total IAA, UA, and CMPF. The other eGFR formulations gave comparably disappointing results with regard to their association to uremic solute concentrations. Relative similarity in R^2 values per solute for the different eGFR values, and the strong disparity in values between solutes, suggest that the differences in R^2 are mainly due to discrepancies in solute handling apart from GFR. **Conclusions:** eGFR is poorly associated with concentrations of all studied uremic toxins in patients with different degrees of CKD, and correlates differently with each individual solute. Hence, eGFR can not be considered representative for evaluating the accumulation of solutes in the course of CKD.

P7 (EI0071)

Role of Killer-Cell Immunoglobulin-Like Receptor and Human Leukocyte Antigen in Kidney Transplantation

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Objectives: Innate immunity represents a new frontier in the field of transplantation. Natural killer (NK) cells in particular have a role as a bridge between the innate and adaptive immunity. Killer-cell immunoglobulin-like receptors (KIRs) belong to a polymorphic family of activating and inhibitory receptors expressed on the surface of NK cells and recognize human leukocyte antigen

(HLA) class I ligands. The aim of this study was to investigate if KIR/HLA compatibility affects renal allograft survival on the long term. **Methods:** We studied 113 patients who received kidney transplant between 1999 and 2005. Eighty-six kidney transplant recipients had a stable renal function, while 26 showed a decrease of their renal function by 20% 5 years post-transplant. The two groups of patients were matched for sex, donor and recipient age, dialysis vintage, cold ischemia time and therapy. All the patients were typed using HLA and KIR-SSO genotyping test. We analyzed the presence of single KIR genes and haplotypes in relation to the decrease of renal function by 20%. Finally we examined all the possible matches/mismatches between KIR genes and known HLA ligands in donor/recipient pairs. **Results:** The presence of the KIR2DS3 gene was associated with a better trend of serum creatinine and MDRD over time ($p < 0.05$), while in the presence of the ligand, the serum creatinine and MDRD trend seems to worsen in the long term. The analysis performed according to whether there was deterioration of renal function or not, showed that the absence of the KIR2DL1 gene is strongly associated with an increase of 20% of the creatinine value at 5 years, suggesting a potential protective effect given by this gene. **Conclusions:** Our data suggest that KIR genes and their respective HLA class I ligands may influence long-term graft outcome after renal transplantation.

P8 (EI0280)

Possibility of Quantum Entanglement Between Artificial Organ, Immune System, and Patient

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Objectives: Analyze the possibility of a quantum entangled between artificial organ (Ao), immune system (Is) and patient (Px). **Methods:** The quantum interaction between artificial organ (Ao), immune system (Is), and patient (Px), can be described with the GHZ quantum formalism by wave function: $\Psi AoIsPx> = 1/\sqrt{2}(|Ao\uparrow Is\uparrow Px\uparrow> + |Ao\downarrow Is\downarrow Px\downarrow>)$, where, by analogy with GHZ quantum model, we could be consider two ($\uparrow$ and $\downarrow$) of the maximally entangled states of three quantum entities ΨAo, ΨIs, ΨPx. **Results:** The wave function $\Psi AoIsPx>$ is produced by maximum entanglement between the various states of the ΨAo, Ψ Is, Ψ Px. Artificial organs could be in a quantum state of functionality $Ao\uparrow$ or nonfunctionality $Ao\downarrow$, immune system could be in a quantum state of nonrejection $Is\uparrow$, or rejection $Is\downarrow$, and the patient could be in a quantum state of wellness $Px\uparrow$, or nonwellness $Px\downarrow$. Now, we could describe this entangled states: $\Psi AoIsPx> = 1/\sqrt{2}(|Ao\uparrow Is\uparrow Px\uparrow> + |Ao\downarrow Is\downarrow Px\downarrow>)$; $\Psi AoIsPx> = 1/\sqrt{2}(|Ao\uparrow Is\uparrow Px\downarrow> + |Ao\downarrow Is\downarrow Px\uparrow>)$; $\Psi AoIsPx> = 1/\sqrt{2}(|Ao\uparrow Is\downarrow Px\uparrow> + |Ao\downarrow Is\uparrow Px\downarrow>)$; $\Psi AoIsPx> = 1/\sqrt{2}(|Ao\uparrow Is\downarrow Px\downarrow> + |Ao\downarrow Is\uparrow Px\uparrow>)$. **Conclusions:** In GHZ-type quantum states, the equation $\Psi AoIsPx> = 1/\sqrt{2}(|Ao\uparrow Is\uparrow Px\uparrow> + |Ao\downarrow Is\downarrow Px\downarrow>)$ could be seen as the best possibility therapeutic in the quantum states after implant. Indeed, artificial organ (Ao), immune system (Is) and patient (Px) are entangled as "functionality" for artificial organ, "non- rejection" for immune system, and "wellness" for patient. The maximally entangled GHZ-type states, could be used to predict that entanglement between artificial organs, immune system, and patient could be therapeutic, where only one maximally quantum entangled state could be therapeutic.

P9 (EI0043)

Posttransplantation Immunologic Monitoring of Renal Allograft Recipients to Differentiate Infections and Graft Rejection

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Objectives: The two major complications after any solid organ transplantation are the graft rejection (a result of immune conflict) and infection processes (bacterial, viral, fungal). Each one requires a very specific and absolutely adequate therapeutic approach: immunosuppression or anti-infective agents. This is the reason, we need a very exact differentiation between both. **Methods:** Our model of immunologic monitoring (IM) includes several highly informative tests to evaluate the dynamic immunoreactivity of the recipients of renal allografts (108 patients, 64 male, 44 female). The T-helper and T-supressor cell activity and the index Th/Ts, the macrophage activity NBT-test, the enzymes SDH, alpha-GPDH, LDH and their quantitative levels, the NK- and K-cell activity, the RBT, the monocyte test for Fc and C3 receptors, the warm and cold antibodies, the TNAB morphologic analysis, as well as the level of immunodefficiency, allow us to determine the immune state of the patients in different phases after transplantation and more important, to predict the forthcoming complications. **Results:** Thus, the proper and exact differentiation diagnosis of graft rejection and infections requires the adequate therapy for each case and eliminates the possible mistakes, such as high-dose immunosuppression when no rejection is detected or anti-infective agents when only the immune conflict is demonstrated. **Conclusions:** Our model of IM allows a precise evaluation of the immunoreactivity of the recipient of renal allografts in any phase after transplantation and can predict the post-transplantation complications.

P10 (EI0108)

Assessment of The Hemoglobin Glycation Applying Mathematical Modeling and Culturing of The Human Erythrocytes In Vitro

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Objectives: Glycated hemoglobin A1c (HbA1c) is the most commonly used parameter characterizing the long-term metabolic control either in a routine diabetes therapy or in experimental treatments aiming at substitution or supplementation of the pancreatic insulin secretion. The objective of the study was to assess ability of a mathematical model of the hemoglobin glycation to reproduce changes of HbA1c occurring in cultures of the human erythrocytes in vitro in response to different glucose concentrations. **Methods:** The model that was used assumed that HbA1c formation in each erythrocyte obeyed first order kinetics in respect to hemoglobin and glucose and that glycation reaction was lasting throughout the whole erythrocyte life span in vivo and was continuing till its apoptosis in vitro. The overall glycation rate and HbA1c in each equal-aged group of erythrocytes was estimated individually for each subject based on the results of continuous glucose monitoring in vivo. Three constant glucose concentrations were applied in the culturing media in vitro (5.2, 10.5 and 15.7 mmol/l). The cultivation lasted for 4–5 weeks. The study group consisted of 10 nondiabetic volunteers (8 females and 2 males). **Results:** The mean difference of HbA1c (MD) predicted by the model and measured in vitro was equal to –0.54% ± 0.62%, –0.30% ± 0.50% and +0.12% ± 0.71% ($p = 0.011$), and the mean absolute difference (MAD) was equal to 0.62% ± 0.54%, 0.56% ± 0.39% and 0.66% ± 0.58% ($p = 0.53$) for three glucose concentrations tested, respectively. Predictions of the model were significantly more accurate during the first 2 weeks of the erythrocytes' cultivation ($p = 0.0000002$). **Conclusions:** The obtained mean values of MD and MAD indicated high ability of the model to reproduce relationship of HbA1c and glucose in vitro. The model used in vitro was identified based on the in vivo glucose data, which confirmed also its applicability under in vivo conditions.

P11 (EI0412)

Blood Pressure, Antihypertensive Treatment, and Graft Survival in Renal Transplant Recipients Using Elderly Living Kidney Donors

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Background: Hypertension is common following renal transplantation and adversely affects graft and patients survival. Strategies for antihypertensive therapy and target blood pressure have not been yet defined. LRT is still predominant in the Balkan countries. The aim of the study is to investigate the role of hypertension on graft survival among the recipients with older kidney donors. **Methods:** We performed 230 LRT in the last 20 years, 90 with the donors older than 65 years (ED). The recipients mean age was 45+6. The standard immunosuppression was used. The Kaplan Meier 5 years graft survival and renal function were analysed and compared with the group of 110 younger donors (mean age 53.4) and their recipients (mean age 32.2-YD). Blood pressure was determined retrospectively from the mean three clinic readings and antihypertensive drugs. Patients were stratified as controlled (CBP < 140/85, n = 170) with one or more antihypertensive drugs and uncontrolled (UBP > 140/85, n = 60). The patients received CCB, ACEI, ABR, BB and diuretics **Results:** The 5 years graft survival rate in the ED was 76% compared with 81% in the YD (ns). The serum creatinine on the end of follow up was 146.04 in ED compared with 123.38 in YD ($p < 0.001$). Five years after transplantation 96% of the transplant recipients in ED received at list 1 while 54% 2 or 3 additional antihypertensive drugs, compared with 85% and 41% of the YD, respectively. The controlled and uncontrolled hypertensive transplant recipients are equally distributed in both ED (CBP = 69%, UBP = 31%) and YD (CBP = 73% and UBP = 27%). **Conclusion:** CBP contributes to the satisfied 5 graft survival and renal function in ED. The authors confirmed the beneficial effect of anti-hypertensive drug treatment on graft function and survival. Therefore, the use of elderly living donors remains a valuable source of kidneys.

P12 (EI0028)

Establishment of Rat Hepatocyte Producing High Mobility Group Box 1 Inhibitor

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Background: One solution for severe hepatic failure may be a hybrid bioartificial liver device containing functioning hepatocytes (Shinoda M. J Surg Res. 2007). High Mobility Group Box 1 (HMGB1) is known as a key mediator in acute liver faulure (Takano K. Shock. 2010). A domain of HMGB1, the A box, competes with HMGB1 for binding receptors and attenuates HMGB1-induced inflammation. In this study, we established rat hepatocyte producing High Mobility Group Box 1 inhibitor, A box. **Materials and methods:** We established three types of adenovirus vectors encoding A box. The vectors included a wild type (type W) and 2 mutant types (type A and type N). We transfected the three types of vectors to primary cultured rat hepatocytes, respectively. The culture supernatant was subjected to western blot analysis for A box protein. The supernatant obtained from the culture with type N was also subjected to an in vitro test of TNF release inhibition from macrophage (RAW 264.7); macrophage was cultured with recombinant HMGB1 or both recombinant HMGB1 and the supernatant containing A box protein. **Results:** Western blot analysis showed a clear expression of A box protein in the culture supernatant of transfected rat hepatocytes in all types of vectors. The expressions were observed from 2 to 4 days after transfection. The expression was much stronger in type A and type N than in type W. TNF release from macrophage was significantly suppressed in the culture of both HMGB1 and A box compared to that of HMGB1 only (401 ± 22 in A box (–) vs 248 ± 16 in A box (+), pg/mL, $p < 0.05$). **Conclusions:** Rat hepatocyte producing HMGB1 A box protein was established. Incorporation of this hepatocyte into a hybrid bioartificial liver is of great interest.

P13 (EI0016)

Chronic Liver Damage Correction by Means of Intracorporeal Bioartificial Liver Unit (Blu), Containing Long-Term Surviving Donor Cells

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Objectives: Working out of BLU for a long time supporting of damaged liver is an actual problem of modern hepatology. This research was undertaken for creation of intracorporeal BLU, containing long-term functioning liver cells (LC) and multipotent mesenchymal stromal cells (MMSC), attached on biodegradable matrix. **Methods:** Hepatic failure was modeled in Wistar rats by using CCl_4 according to the standard scheme within 6 weeks. Adult healthy Wistar rats were used as donors of MMSC and LC. MMSC were obtained by standard procedure and cultivated during 10 days in DMEM with additions. Isolated LC were obtained by a standard procedure with 0,12% collagenase solution. Suspension of LC and MMSC were mixed, seeded on biodegradable matrix *Sphero* GEL as $2{,}0–4{,}0 \times 10^6$ cells/cm^3, and cocultivated within 3 days. Matrixes with attached LC and MMSC as BLU were transplantated into damaged livers. Dynamics of hepatic failure reduction, and survival LC were investigated in 30, 60, 90 and 180 days after BLU transplantation. **Results:** Cell viability measured after isolation was: LC $-76 \pm 4\%$, MMSC $-94 \pm 2\%$. On the 30th day after BLU transplantation GPT, GOT, ALP returned to normal levels. In control rats—the same indices returned to normal levels more than in 6 months. Viable and high proliferative activity of transplanted LC were determined more than in 180 days after BLU transplantation. **Conclusions:** Our studies asserted the long time survival of LC and MMSC attached on matrixes and transplantated into damaged livers. We consider that the present data are an important step toward clinical application of intracorporeal BLU as a bridge to OLT.

P14 (EI0011)

The Ex Vivo Normothermic Perfused Liver-Kidney Model: An Improvement of The Circuit's Biochemical and Acid-Base Environment

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Objectives: The *ex-vivo* liver perfused model allows a better and unequivocal analysis of changes obtained by dissociating the organ from the extrinsic regulatory mechanisms. We now analyse the influence on the biochemical environment obtained with the addition of a kidney to the circuit. **Methods:** Eight livers were harvested from female pigs and perfused for 6 hours. In five additional experiments a kidney was also harvested and connected in parallel. The extracorporeal circuits included a centrifugal pump, heat exchanger, and oxygenator. Hourly arterial blood gases were collected to analyse glucose, PH, bicarbonate, base excess, urea, creatinine, sodium, potassium. Primary endpoint of the study was to evaluate the influence of the kidney on glucose, PH and electrolytes levels. **Results:** In the liver-kidney circuit all parameters examined had significant lower values compared to the liver circuit only. This was particularly evident for glucose values where a normoglycemia was reached at the end of the perfusion and for PH and electrolytes that were maintained on steady levels. **Conclusions:** The addition of the kidney to the circuit provides a better biochemical environment by filtering the excess products continuously released from the metabolisms. This could open the way for future experimental *ex-vivo* models that require a strict balance of these elements.

P15 (EI0135)

Indications and Limits of The Replacement Therapy of The Liver Detoxifying Function By Mars (Molecular Adsorbent Recirculating System) In The Treatment of Acute-On-Chronic Liver Failure (ACLF)

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Objectives: To assess indications and limits of MARS in ACLF treatment **Methods:** 98 patients affected by ACLF, secondary to different liver diseases, 80 with INR values <3 (group A) and 18 with INR values ≥3 (group B,) have been treated with MARS (5 h daily sessions, blood flow 220 ± 20 ml/min, albumin 150 ml/min, sessions from 2 to 7, according to the patient needs). All the measured liver function parameters were not significantly different between the two groups. 42 patients lamented severe pruritus and showed scratching skin lesions before the treatment. **Results:** At the end of each treatment total bilirubin, bile acids and ammonia, fell of $28 \pm 9\%$, $40 \pm 8\%$, $54 \pm 14\%$ respectively, with post-treatment rebound variable in each subject. After a cycle of MARS treatments liver function tests improved, total bilirubin, bile acids, ammonia, alkaline phosphatase and INR values significantly decreased in group A, while no improvement was observed in group B. Pruritus disappeared in all the patients after the third MARS treatment. **Conclusions:** These observations suggest that the severe lack of coagulation factors, which reflects the end-stage liver failure, is the main conditions indicating the exclusion of MARS treatment. The treatment is not absolutely contra-indicated even if other parameters are seriously altered. In fact its efficacy is strictly related to the potential recovery of liver function, independently of primary liver disease.

P16 (EI0125)

Obtaining Pig Liver Scaffolds: Development and First Results of a Decellularization Model

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Objectives: Based on previous experiences published on decellularization of small animals' solid organs, in order to obtain bioenginered scaffolds for posterior recellularization, we aimed to develop a model to reproduce the aforesaid results in large mammals, as a previous step towards the translation to human liver. To achieve this goal, we propose a pressure controlled perfusion system for isolated organs with continuous monitoring. Using a detergent solution previously proved effective, we intend to obtain ten specimens. **Methods:** We harvested ten specimens of minipig liver, using the same technique as in humans, with cold perfusion of preserving solution via portal vein and through hepatic artery via aorta. After the back table preparation, the organ was connected to the pressure controlled perfusion system, to continuously infuse a SDS based decellularizating solution for 24 hours. The system included a remote controlled pump with a pressure sensor all connected to a computer with the controlling software, developed at our institution. Once the decellularization procedure was finished, we checked the integrity of the vascular and biliary anatomy with radioscopy. A complete histological study was then performed, including optical and electron microscopy. Finally we deter-

mined the DNA residue present in the scaffold to assess the completeness of the lavage. **Results and Discussion:** After a preliminary experience with three organs, we were able to establish a method for the decellularization. With this model, we performed seven more procedures with porcine livers, which were all completely devoid of cells and comparable in terms of macroscopic appearance, histological and anatomical analysis and DNA residue. **Conclusion:** We have developed a reproducible model for decellularizating large mammals' organs to use as bioengineered scaffolds for recellularization, which is a feasible initial step towards the aim of creating bioartificial organs for transplantation in humans.

P17 (EI0058)

Membranes with Embedded Sorbents for An Artificial Kidney

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Objectives: Chronic kidney failure requires an artificial kidney treatment called hemodialysis. However, not all uremic toxins can be removed by dialysis. Sorbents can be used for purification of blood, but this is often limited because of poor hemocompatibility. In this work, we propose a new concept to improve blood purification as well as the hemocompatibility when sorbents are used. We develop mixed matrix membranes (MMM) which consist of adsorptive particles embedded into a porous polymer matrix. The MMM can combine diffusion and adsorption in one step. To enhance the hemocompatibility of the MMM, a particle free layer is introduced on the blood contacting side of MMM, and several matrix materials are tested. **Methods:** Porous MMM with activated carbon particles and a particle free blood contacting layer were prepared by co-casting and spinning and subsequent phase separation. Several co-polymers with enhanced hemocompatibility as well as polyethersulfone were used as matrix materials. The membranes were characterized by scanning electron microscopy and adsorption experiments and screened for hemocompatibility. **Results:** Double layer membranes with a homogenous distribution of carbon particles were developed. Creatinine was used as model blood toxin. The experiments show that creatinine diffuses through and adsorbs onto the MMM at the same time. Markedly, no quick particle saturation occurs. The contribution of the removal by adsorption is more than 80% of the total creatinine removal after 7 hours. The use of copolymers with hydrophilic and hydrophobic parts as matrix material leads to membranes with increased hemocompatibility. **Conclusions:** This study shows proof of principle for MMM as an artificial kidney. MMM remove toxins via both diffusion as well as adsorption in one step. Our concept is versatile since we can combine various matrix materials with improved hemocompatibility and various sorbents for removal of toxins from blood. The DutchKidneyFoundation is acknowledged for financial support.

P18 (EI0234)

Microbubbles of Air During Haemodialysis- Negligible for the Patient?

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Objectives: The symposium covers information about presence of microbubbles in haemodialysis. Physical basis, technical considerations and regulations that will be presented by Hans-Dietrich Polaschegg (Koestenberg, Austria). Per Jonsson (Umea, Sweden) will cover experimental projects that were focused to evaluate presence of of microbubbles air as contamination in the in vitro setting and some experiences. Those data and techniques were further used in *in vitro* testing of prevailing materials and basis for initial clinical studies on chronic haemodialysis sessions (Bernd Stegmayr, Umea, Sweden). The symposium will thereafter focus on more clinical studies to investigate various clinical settings in relation to microbubble exposure. This will be presented by Ulf Forsberg (Skellefteå, Sweden). The importance of microbubbles of air may be questioned. Therefore post mortem investigation were performed of haemodialysis patients. These data will be presented by Thomas Brännström (Umea, Sweden). The symposium will end with time for discussion.

P19 (EI0160)

Nanoparticles of Ca; Mg,Ca; and Zn, Ca Hydroxyapatite as A Component of Ferromagnetic Polymer Matrix

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Objectives: Ca; Mg,Ca and Zn,Ca; hydroxyapatite (HAP)-nanoparticles are proposed as a component of 3D polymer matrix. Moreover, the incorporation of magnetic particles enables cell manipulation by an externally applied magnetic field. The adhesiveness and magnetic properties of matrix materials as one of the major conditions for a surviving of cells is considered. Process of forming the most suitable matrixes for cells in system polymer—HAP-ferromagnetic is studied. **Methods:** Ca_{10}; Mg_1Ca_9 and Zn_1Ca_9 $(Po_4)_6$ $(OH)_2$ [HAP] particles are obtained by co-precipitation method in water solutions of salts and then dispersed in melt of polyethylene The analysis of matrixes is carry out by XRD, FTIR. The adhesive properties of matrix are studied using the estimation method of bond strength with metals at scaling. The measuring in magnetic field (2,5 kE) carry out using magnetometer. The estimation of adhesion and growth of cells to surface of matrixes carried out with use of a light microscopy, TEM, SEM and fluorescent microscopy. The morphology and viability of fibroblasts cells and cells of a hepatoma of human HepG2 on the surface of matrixes are studied. **Results:** The samples possess an amorphous structure of HAP, or incompletely crystallized with additional peaks of polymer. The size of nanoparticles is in the range from 10.0 to 60.0 nm.The adhesiveness of matrix to metals varies from 2.0 to 14.0 kg/cm. Magnetization of matrixes varies from 7.5 to 23 emu and concentration of magnetic phase from 4.0 to 12.0%. Visualization of cells on surface of the matrix allows concluded that all samples are not toxic. The adhesiveness of cells to a surface is very different. The characteristic properties of cells are depended on the composition of matrix. **Conclusions:** The received data have practical significance for reconstructive surgery and transplantation.

P20 (EI0260)

Preparation and Characterization of Water-Soluble C60/Silk Fibroin Nanocomposite for Cartilage Regeneration Application

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Objectives: Studies have showed the role of water-soluble C_{60} in protection of articular cartilage against progression of osteoarthritis. Silk fibroin based scaffolds also have been explored in cartilage or bone tissue engineering for years. Among them, aqueous derived silk fibroin scaffolds prepared via salt-leaching approach acted as promising candidates in tissue engineering application. However, salt-leached silk fibroin scaffolds derived from highly concentrated aqueous silk fibroin solutions haven't been reported. In this study, the aim is to prepare aqueous derived salt leached silk fibroin scaffolds with improved mechanical properties. Furthermore, these novel scaffolds will combine with water-soluble C_{60} to generate nanocomposites for cartilage regeneration. **Methods:** Silk fibroin was firstly extracted from silkworm *Bombyx mori* by degumming in sodium carbonate solution. And then, the silk fibroin was dissolved in lithium bromide solution and dialyzed against distilled water. By its turn, concentrated silk fibroin solution was achieved by dialysis against poly(ethylene glycol) solution. Salt-leached silk fibroin porous scaffolds were prepared by the addition of sodium chloride particles into the silk fibroin solution. Water-soluble C_{60} was prepared via acid treatment and subsequent methacrylation [1]. The morphology, microstructure, mechanical properties and the cytotoxicity properties of the silk fibroin scaffolds. The modified C_{60} was characterized by FTIR and NMR. **Results:** The mechanical properties of the silk fibroin scaffolds improved dramatically when prepared with high concentration silk fibroin solutions. The FTIR and NMR spectra showed that the carboxyl group and methacrylate group was successfully grafted with C_{60}. **Conclusions:** A novel salt-leached silk fibroin scaffold was generated via using highly concentrated silk fibroin solutions. The water-soluble C_{60} can be prepared via chemical modification. It is expected that the preparation of water-soluble C_{60}/silk nanocomposite could bring new insights in cartilage regeneration.

P21 (EI0092)

Mscs Proliferation and Osteogenic Differentiation on 2D and 3D Pcl Nanofibrous Scaffolds

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Objectives: Nanofibers appear to be the ideal material for scaffold development in regenerative medicine. On the other hand electrospun nanofibres form two-dimensional (2D) net. Cells can proliferate only until confluence is reached. In this work advantages of extensive surface and three-dimensional (3D) scaffolds were mixed together to prepare 3D electrospun nanofiber scaffold. **Methods:** Two different samples were prepared from PCL using electrospinning in the same conditions, only collector was different. In the case of 2D nanofibers, collector was plain, to prepare 3D samples, structured collector was used. Structure of samples was visualized by scanning electron microscopy

(SEM). Samples were seeded with 9×10^5 pig MSCs and cultured for 21 days. For detection of MSCs adhesion, spreading area of cell was measured. Cells were stained using DiOC and visualized by confocal microscopy. Proliferation and viability were detected using MTT assay and live/dead staining with subsequent confocal microscopy visualization. Osteogenic differentiation was investigated with real-time PCR analysis; osteocalcin (OC) and bone sialoprotein (BS) were used as osteogenic markers. **Results:** Both, structured and nonstructured samples were successfully prepared. SEM shows that samples prepared using structured collector have 3D structure. MSCs adhered well on both, 2D and 3D scaffolds. On 3D scaffolds spreading area was slightly higher. Proliferation was higher on 3D nanofiber scaffolds on 21 day. Better proliferation was confirmed by live/dead staining and confocal microscopy. OC and BS were used as markers for detection of late osteogenic differentiation. In 2D and 3D samples osteogenic markers were present, whereas higher amount of both markers were shown on 3D scaffold on 21 day. **Conclusions:** It was shown that 3D structured PCL nanofibers are promising for purposes of tissue engineering. Acknowledgement: The Grant Agency of the Charles University (grant No., 330611), Grant Agency of Czech Republic (grant No. P304/10/1307).

P22 (EI0149)

Plasma Exchange in Anca Associated Vasculitis with Severe Renal Involvement

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Objectives: Systemic vasculitis associated with autoantibodies to neutrophil cytoplasmic antigens (ANCA) is the most frequent cause of rapidly progressive glomerulonephritis. Renal failure at presentation carries an increased risk for chronic kidney disease (CKD) and death despite immunosuppressive therapy. Early and accurate diagnosis and aggressive treatment are essential to optimizing outcomes while avoiding unnecessary immunosuppressive therapy. **Methods:** This study investigated the role of plasma exchange in the achievement of renal recovery in patients who presented a serum creatinine 500 µmol/L. We present here three patients treated in ICU at University Clinic of Nephrology in Skopje, with confirmed diagnosis of ANCA vasculitis associated with respiratory symptoms and a renal affection manifested as glomerulonephritis. **Results:** All patients had diagnosis of ANCA associated vasculitis confirmed by renal biopsy and serum creatinine >500 µmol/L. Initial hospital admission was marked by serious respiratory symptoms with development of important deterioration of renal function as well as anemia and hypoproteinemia. Dialysis treatment was introduced as well as immunosuppressive therapy with prednisolone and cyclophosphamide by EUVAST recommendations. Plasma exchange was also introduced in at least 9 sessions per patient. This together with immunosuppressive therapy resulted in a disappearance of signs and symptoms of systemic inflammation and in an important improvement of respiratory symptoms and moderate improvement of kidney function. Patients were discontinued from dialysis and at a point of 3 months after admission all patients were dialysis independent. **Conclusions:** In patients with clinically and histologicaly confirmed ANCA associated vasculitis, plasma exchange together with recommended immunosuppressive therapy can increase the rate of renal recovery and should be considered as an effective adjunctive modality of treatment.

Smart and Responsive Biomaterials

P23 (EI0348)

Synthesis of Poly (Acrylamide-Co-Itaconic Acid) Hydrogels and Their Interactions with Calcium Ions and Antibiotic with Potentials Applications in Orthopedic Medicine

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Objectives: The need to repair bone defects is a significant problem faced in orthopedic medicine. Biomaterials, such as polymers are used in bone tissue engineering. Calcium phosphates, used clinically in orthopedic surgery, have attracted attention as bone substitutes, due to their good biocompatibility and osteointegrative properties[1]. In this work hydrogels obtained from poly (acrylamide) and poly (acrylamide-co-itaconic acid) were synthesized and characterized to determine their capacity for calcium absorption and antibiotic interaction. **Methods:** Hydrogels copolymers of acrylamide (AAm)/itaconic acid (AI), cross-linked with N,N-methylenbisacrylamide were synthesized, from AAm/AI with fed proportions 90/10 and 80/20. The hydrogels swelling degree was measured in deionized water and in calcium salts solutions. Also was measured in antibiotic (Tygacil). Inductively coupled plasma optical emission spectrometer (ICP-OES) was used to analyze the aqueous calcium solutions. Hydrogels were observed by scanning electron microscope (SEM) (Hitachi S-800). By SEM was also analyzed the calcium absorbed in the hydrogels structure by EDX. **Results:** In poly(AAm-co-AI), hydrogel the equilibrium swelling measured in aqueous calcium solutions decreased between 5 to 10% compared with the swelling in pure water. ICP-OES results indicates that poly (AAm-co-AI) hydrogels have an efficient absorption toward calcium ions (13,69 mg Ca^{2+}/g hydrogel), while poly(acrylamide) only absorbed 3.83 mg Ca^{2+}/g hydrogel; after they were submerged for 24 h. The poly(acrylamide) hydrogels morphology evaluated by scanning electron microscopy revealed pore dimension ranging from 210 nm to 1430 nm, that increased to 6900 nm when it was copolymerized with the itaconic acid. **Conclusions:** The results show evidence of an efficient reception of Ca^{2+} ions by the polymers, especially poly (AAm-co-AI) hydrogels containing 20% of AI. The porosity morphology revealed interconnected pores, which is important because it has been reported that an open porosity would be necessary to ensure rapid colonization of the implant with blood vessels and bone cells.

P24 (EI0178)

Development of An Electrochemical Sensor for Heparin in Blood Using Molecularly Imprinted Polymer

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Objectives: Inhibition of blood-coagulation is very important for safe extracorporeal circulation. However, suitable methodology for monitoring anticoagulant in blood is yet to be established. The purpose of this study is development of a sensor for level of heparin, which is the well-used anticoagulant, using an electrode grafted with molecularly imprinted polymer (MIP). **Methods:** A photoinitiator of radical polymerization was introduced on indium-tin oxide (ITO) covalently. Heparin and (2-mathacryloxyethyl) trimethylammonium chloride and acrylamide was dissolved in water. Methylenebisacrylamide was dissolved in dimethylformamide. The initiator-immobilized ITO was soaked in the mixture of the solutions and was irradiated by UV-lump for graft polymerization. The treated electrode was rinsed by water to obtain an electrode grafted with heparin-imprinted polymer (HIP). Another electrode grafted with nonimprinted polymer (NIP) was prepared by the same procedure except heparin was omitted. A traditional cyclic voltammetry of ferrocyanide was performed with the polymer-grafted electrode in physiological salt solution diluting bovine blood. The effect of heparin in blood on the oxidation current at the voltammetry was observed. **Results:** The anodic current at the HIP electrode was increased by the presence of heparin in the blood (4 unit/mL). The change of current by heparin was reversible. However, the current at the NIP electrode was insensitive to the heparin. The current change at HIP was probably due to specific binding between heparin and the imprinted site which was created during the polymerization. Then the porosity of the HIP layer was increased responding to the specific binding and accessibility of the ferrocyanide toward electrode was enhanced. **Conclusions:** The concentration of the heparin in the blood can be detected by voltammetry using an electrode grafted with heparin-imprinted polymer. The procedure is very simple. Then the method is feasible for monitoring of heparin in the circulated blood.

P25 (EI0311)

Preparation of Bioinspired Magnetic Responsive Hydrogel Particles for Biomedical Applications Using Superhydrophobic Substrates

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Objectives In tissue engineering and regenerative strategies the number of cells extracted from the patient are not enough for the therapy. In this context, polymeric microspheres have been used as supports for the expansion of cells. Recently inspired by the rolling water droplet on a lotus leaf, a novel methodology on preparing hydrogel and polymeric spheres was developed using superhydrophobic substrates. In this contribution, magnetic responsive hydrogel beads were prepared by this innovated method; furthermore, the cells attachment was investigated on these hydrogel beads before and after plasma treatment. The introduction of magnetic microparticles during sphere production permits the isolation of the particles from the culture medium simply by using an external magnetic field. Methods Polystyrene superhydrophobic substrates were prepared by a simple phase inversion method. Magnetic microparticles of Fe3O4 were introduced during hydrogel sphere production process. After frozen dried, the beads were further treated by Argon plasma. Cells attachment was investigated on both treated and untreated ones. Results and Conclusions

Magnetic responsive chitosan hydrogel spheres crossing-linked by genipin were hardened on the superhydrophobic surfaces. Upon plasma treatment, cell attachment onto the beads surface was improved as compared to the untreated ones. The chitosan beads could move throughout the liquid medium by the action of an external magnetic field. After extracting the particles from the medium the cells could be detached from such supports by the action of trypsin and the particle could be used again.The extracted cells were found to maintain their viability. In conclusion, magnetic responsive hydrogel beads could be prepared by using superhydrophobic substrates

P26 (EI0303)

Hr-Mas Nmr Spectroscopy as Efficient Tool To Characterize Crosslinked Hydrogels

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Objectives: In the present work, high-resolution magic angle spinning (hr-MAS) NMR spectroscopy is applied as a straightforward nondestructive technique to quantify unreacted methacrylamide functionalities in cross-linked gelatin hydrogels. **Methods:** 10 w/v% cross-linked methacrylamide-modified gelatin (gel-MOD) hydrogel films were prepared and lyophilised, followed by resuspension in D_2O. The effect of the photo-initiator concentration (Irgacure 2959, 0.5–10 mol%) and the applied UV irradiation time (5–30 minutes) on the consumption of methacrylamide moieties was evaluated using hr-MAS NMR spectroscopy. **Results:** The results (data not shown) indicate that a critical amount of 2 mol% photo-initiator is required to obtain a significant amount of methacrylamide-crosslinking. Upon increasing either the photo-initiator concentration (2–10 mol%) or the UV irradiation time (5–60 min), the percentage of reacted methacrylamides increased significantly ($P < 0.05$). Interestingly, it can be observed that even at a UV irradiation time of 1 hour and a photo-initiator concentration of 10 mol%, only 40% of the methacrylamide side groups have reacted. In a final part of this work, rheological measurements were performed to correlate the mechanical properties of the hydrogels developed with the cross-link efficiency obtained from hrMAS NMR. Increasing either the photo-initiator concentration or the UV irradiation time, leads to an increased storage modulus. In addition, the mechanical data indicated that both the storage and loss moduli display a pronounced plateau value in the frequency region studied. Moreover, G′ is about two orders of magnitude higher than G″, which is indicative for the formation of a well established network. **Conclusions:** We can conclude that hr-MAS NMR spectroscopy is a suitable, nondestructive and straightforward tool to evaluate absolute hydrogel crosslinking efficiencies. Although the technique was only applied for gelatin-based hydrogels, we are at present investigating the applicability to other hydrogels including vinyl functionalised Pluronic based systems.

P27 (EI0205)

Enhanced Function and Adhesion Mechanism of Human Bone Marrow Cells on Functionalized Carbon Nanotubes

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Objectives: Carbon nanotubes (CNTs) have attracted much attention as a biomaterial with interesting potential applications. The disadvantage of insolubility has been overcome by functionalization of CNTs surface. In this study, human bone marrow stromal cell (HBMSC) adhesion, proliferation, and differentiation, cultured on multiwalled CNTs, either pristine or amino-, carboxy- and hydroxy-functionalized, were investigated. Preliminary results on integrin-mediated adhesion mechanisms were also obtained. **Methods:** Functionalized CNTs were synthesized according to well established methods and were fully characterized with TGA and Raman analysis. The HBMSCs were routinely cultured human in osteogenic medium. Cells of second to forth passage were seeded on the materials. Morphology and adhesion were evaluated by SEM after 1 and 3 days, proliferation was estimated by DAPI staining after 3 and 7 days and differentiation through alkaline phosphatase activity (ALP). To screen which integrins were responsible for attachment of the cells, monoclonal antibodies against integrin subunit β_1 were incubated with the cells for 30 min at 37°C, prior to seeding on the CNTs surfaces. **Results:** SEM images display that HBMSCs attach and spread well on all CNTs surfaces without differences in their morphology. Proliferation was highest on amino-functionalized CNTs, but not significantly different from the other surfaces. However, proliferation on the control (tissue culture plastic) was 3-fold. The highest expression of ALP activity was on hydroxy-functionalized CNTs and the lowest on pristine. After 3 days of culture, the ALP expression on CNT surfaces was approximately 70% of the control, whereas after 7 days, it was 4-fold of the control. The blocking of integrin subunit β_1 with monoclonal antibodies resulted in decrease of the adhesion percentage to pristine CNTs at 40–50%. **Conclusions:** It is possible that nanomaterials, whose structural features resemble those of natural tissue enhance cell function and CNTs can be used as orthopaedic biomaterials.

P28 (EI0124)

Physical Crosslinking of Gelatin: A Supramolecular Approach to Biomaterial

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Objectives: Key properties that a biomaterial should address include elastic properties close to the substituted tissue, specific adhesion epitopes, and tailorable degradability. A knowledge-based approach formed the basis for investigating the systematic variation of material properties of gelatin by introducing functional groups derived from tyrosine for enabling π-π interactions as well as hydrogen bonds to form stable physically crosslinked networks. **Methods:** Gelatin was functionalized with desaminotyrosine (DAT) or Desaminotyrosyl-tyrosine, (DATT). Atomistic molecular models of pure and functionalized gelatin with 0.8 wt.-% or 25 wt.-% water content were constructed using Material Studio (Accelrys) and submitted to the Amorphous Cell module to create bulk packing systems. The dynamic behaviour, structural, and mechanical properties were investigated by analyzing free volume distribution, solubility parameters, elastic properties, and aggregation phenomena. The functionalized gelatins were synthesized by coupling of the free carboxylic acid groups of DAT(T) to the amino groups of gelatin and the materials were characterized by tensile tests, TM-DSC, swelling experiments, and WAXS. **Results:** The simulations predicted an increasing number of aromatic functions attached to the gelatin chain leading to an increase in the number of physical net-points. In the synthesis, about 80 mol.-% of all amino groups were functionalized with DAT(T). Increasing the number of aromatic groups attached to the gelatin chain resulted in suppression of helix formation and decreased the swelling degree. Mechanical properties (Young's modulus, elongation at break, and maximum tensile strength) of the gels at equilibrium swelling increased with the number of introduced aromatic groups. **Conclusions:** Distinct tailoring of material properties was achieved for a biopolymer by only small changes in molecular structure of gelatin. The approach of molecular modeling of gelatin as bulk material permits to analyze structural features of functionalized materials and can be used as predictive tool in the design of new biopolymer-based materials.

P29 (EI0336)

Temperature-Responsive Microcapsules Prepared by Nanostructured Multilayers of Chitosan and An Elastin-Like Recombinamer for The Controlled Release of Therapeutic Molecules

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Objectives: Polyelectrolyte vesicles using layer-by-layer (LbL) were recently introduced for the encapsulation of therapeutic molecules. This work presents multilayered microcapsules of chitosan and a temperature-responsive elastin-like recombinamer (ELR) as a novel drug delivery system. The release of a pre-loaded model protein was studied at distinct temperatures and number of layers to evaluate the permeability of these structures and their potential as tunable drug delivery devices. **Methods:** Sacrificial $CaCO_3$ microparticles were prepared by co-precipitation of Na_2CO_3 and $CaCl_2$ in a FITC-BSA solution under heavy stirring. LbL coating was performed by incubation with chitosan or ELR solutions, with a rinsing step in between. Capsules with 1, 3 and 5 bilayers were made. The $CaCO_3$ cores were chelated using EDTA. The capsules were suspended in PBS at 25 and 37°C and samples were taken every 24 hours for fluorescence measurements, during 14 days. **Results:** At both temperatures, cumulative release was higher for capsules with 1 bilayer, evidencing the role played by the capsules architecture in their permeability. The release kinetics among each temperature was also different: the BSA quantity released was higher at 25°C than at 37°C. Considering the case of a simple bilayer, in the former the cumulative release reaches 80%, while in the latter only 50% of the encapsulated protein is released. This result shows the effect of temperature in polyelectrolyte structures, namely when temperature-responsive materials like ELRs are used. **Conclusions:** Multilayered microcapsules based on chitosan and an ELR were studied as drug delivery vessels. Distinct release profiles of pre-loaded BSA at different temperatures and layer numbers demonstrated the influence of the capsules architecture and composition: more quantity of BSA is released for capsules with fewer layers and lower temperatures. These microcapsules have potential for tunable drug release in tissue engineering applications by means of design changes.

Engineering for Cardiac Assist Devices

P30 (EI0380)

Cfd Analysis of The Blood Flow in A Hollow Fiber Membrane Oxygenator With Multiple Passageways

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Objectives: This work presents a 3D computational fluid dynamics (CFD) approach for modeling blood flow in a state-of-the-art hollow fiber membrane oxygenator with integrated heat exchanger. During extra-corporeal circulation blood needs to be heated and oxygenated before being reinfused into the patient. Optimal gas exchange and minimal pressure drops are two basic requirements in the design of membrane oxygenators. In the present study, the effects of multiple passageways to enhance blood oxygenation within the device were assessed through a CFD analysis. **Methods:** The fluid volume inside the device was discretized with about 2.5 million elements. Blood was modeled as an incompressible Newtonian fluid with viscosity $\mu = 3.0$ mPa·s and density $\rho = 1060$ kg/m^3. A blood flow of 4 l/min at the inlet section gave rise to nonlaminar inflow conditions (Re = 2880). Hence, the viscous k-ω two-equations turbulence model with low Reynolds number corrections was adopted. The heat exchanger and oxygenator regions were modeled as porous media with permeability values obtained from experimental tests. Laminar flow regime was assumed in those regions. CFD simulations were performed using the commercial software ANSYS FLUENT, **Results:** Computational results were post-processed to extract the flow velocity pattern, the potential stagnation areas and the pressure drops. The shape of the oxygenator ensured a good intermixing of flow thanks to the multiple passages of blood inside the fiber bundle. The overall pressure drop was equal to 200 mm Hg. High local pressure gradients, originating from high flow concentrations in collecting and distributing regions, were detected. **Conclusions:** The CFD-aided analysis allowed to evaluate advantages and drawbacks of the device geometry. The tortuous pattern of blood inside the oxygenator may be an effective strategy to enhance mass and heat transfer within the device, by allowing a multiple blood crossflow within the fibre bundle at low velocities and limiting the pressure drops.

P31 (EI0252)

Transcutaneous Energy Transfer System Tet: In Vitro and In Vivo Validation

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Objectives: Percutaneous drivelines cause infections and technical problems. To minimize complications and increase patient's mobility, a transcutaneous energy- and data transfer system is to be developed with high tolerance of transmission and a convenient external carrier system. **Methods:** The inductive TET includes two coreless coils (D = 60 mm) with external and internal control units equipped with accumulators. An integrated controller provides telemetry data processing and control of the implant. Wireless data transfer is enabled by using RF transmission and a proprietary protocol. The performance is verified in a body simulator in vitro and in acute animal studies (pigs, n = 4). A positioning assistance is developed for exact placement of the external coil. A carrier system for the external components is designed as a flat textile backpack in which the external transmitter coil is integrated. The carrier system is verified in a study with VAD patients. **Results:** The developed TET is able to transmit up to approx. 25 Watt through the tissue. Bi-directional data communication is improved to a rate of 500 kbits/sec, were the external receiver is allowed to be up to 3 m distant to the patient. The maximum efficiency of the system is approx. 83% at 15 mm distance between the coils and 79% at 25 mm distance. Displacement of the coils up to 20 mm reduces the efficiency up to 15% and leads to a warming of the external transmitter electronic. No warming is measured between the coils and the implanted components under any operating condition. The positioning system enables easy alignment of the external coil with an accuracy of 1,6 mm. **Conclusions:** The TET shows reliable transmission at horizontal and vertical displacements up to 35 mm. Transmitted energy is automatically adapted to the demand of the implanted device. Twisting of the flexible coils did not influence the transmission appreciable.

P32 (EI0167)

Development of a Portable Pneumatic Driver for The Whole Range of Berlin Heart Excor Blood Pumps

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Objectives: Paracorporeal, pneumatically driven VADs are preferably used for biventricular adult support as well as for pediatric patients. For the EXCOR System (Berlin Heart GmbH, Germany), a new pneumatic driver that combines the performance and versatility of the existing stationary driver with the flexibility of the existing mobile driver is currently under development. **Methods:** The new driver uses the reliable and proven pneumatics technology of the EXCOR mobile driver. Two piston pumps operate in synchronism with the blood pumps. The piston of each pump is driven by an electric motor via a ball-screw. A closed pneumatic system was chosen to optimize the system efficiency. A control system regulates the pneumatic pressure and blood flow waveform, emulates the Frank-Starling behavior, synchronizes both blood pumps and ensures optimal adjustment of the enclosed air mass. An emergency mode of operation is provided by a fault-tolerant embedded computing system in combination with a dedicated crossover valve without unduly increasing the system's complexity and weight. Special attention has been paid to easy handling and a clear alarm and message structure. The driver is mounted on a cart and is equipped with two easily exchangeable batteries, a mains power adapter and a car power adapter. **Results:** At 8 kg the new driver is lighter than the EXCOR mobile driver. It supports blood pumps in the range of 10 ml to 80 ml. The power consumption for biventricular adult support is 20 W and less for pediatric support. The batteries guarantee cordless operation for at least 8 hours. Bench tests have demonstrated correct performance with respect to preload sensitivity and flow profile. **Conclusions:** The new pneumatic driver is able to drive all sizes of EXCOR blood pumps. Its small dimensions, low weight and new design make it suitable for stationary as well as portable use.

P33 (EI0148)

The Continuous Flow LVAD With Native Heart Load Control System (NHLCS) for Bridge to Recovery Could Control The Coronary Flow and Myocardial Oxygen Consumption in Acute Heart Failure Model

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Objectives: A novel control system for continuous flow LVAD has been developed for bridge to recovery. We have reported that the amount of coronary flow and myocardial oxygen consumption (MVO2) could be controlled by changing its rotation speed in synchronization with the native cardiac cycle, in normal heart models. We will confirm whether the coronary flow and MVO2 can be controlled by the NHLCS under acute heart failure condition. **Methods:** Ten adult goats (61.4 ± 12.6 kg) with acute LV dysfunction due to coronary microsphere embolization (50 μm, 0.42 ± 0.22 million) to left anterior descending artery were used for the experiment. The continuous flow LVAD (EVAHEART) was installed via left thoracotomy. Blood uptake was from Apex and return it to descending aorta. Ascending aortic flow, pump flow, coronary flow of the left main trunk were monitored. LV volume and pressure were also monitored. We performed 4mode, Circuit clamp (no support), Continuous (constant rotation), Counter pulse (increase rotation in diastole), Co pulse (increase rotation in systole) with the 100% bypass rate. **Results:** The amount of coronary flow in counterpulse mode was proved to be significantly increased than any other modes. (Continuous/ Counterpulse/Copulse: 114.0 ± 2.7/121.8 ± 2.7/101.6 ± 2.7 ($p < 0.05$)) We also find out that MVO2 was decreased in counter pulse mode and increased in Copulse mode compared with continuous mode. (Continuous/ Counterpulse/Copulse: 79.7 ± 13.9/70.2 ± 19.2/88.5 ± 13.8 ($p < 0.05$)) (circuit-clamp mode = 100) **Conclusions:** We have showed the possibility for changing the coronary flow and MVO2(=heart load) by controlling the rotation of continuous flow LVAD. It may be suitable to use counterpulse mode for support mode by its large coronary flow and small MVO2. And it may be also suitable to use copulse mode for heart training mode by its large MVO2. Using this novel control system(NHLCS) flexibly according to patient circumstances, may contribute to the bridge to recovery therapy for patient with LVAD.

P34 (EI0227)

Development of An Innovative Mock Circulatory Loop for VAD Testing

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Objectives: In this paper a novel approach for the design of a Mock Circulatory Loop (MCL) is presented. This modular MCL concept is composed of active components only that are integrated into a Hardware-In-The-Loop (HIL) simulation. Hence, various conditions of a patient and transitions between conditions can be simulated with the MCL. **Methods:** At present the newly developed MCL consists of two modules. The modules are composed of active components enabling optimal controllability of the system. In its current configuration it can be used to test ventricular assist devices. As vessels are typically described as a combination of a resistance and a compliance the modules are composed of gear pumps and metal bellows that are actuated from voice coil actuators. They represent the vessel's resistances and compliances. The inertance of larger vessels was neglected. Furthermore, the MCL can mimic the heart as well, because ventricles are usually modelled as variable compliances. Since the MCL is part of a HIL-simulator it is controlled by a dSpace DS1103-system that allows real-time simulation of the cardiac circulation. A

software simulation of the cardiovascular system was used to generate setpoint values for the modules. **Results:** At first, measurements with the components of a module were performed in order to verify compliance with the prior defined requirements. Afterwards, simulations of the aortic and the left ventricular pressure were used as setpoint values for the modules. Both modules successfully met the requirements and were capable to trace the desired hemodynamic conditions. **Conclusions:** The results demonstrate the feasibility of the test stand. In a next step the MCL will be enhanced by two additional modules for total artificial heart testing. **Acknowledgments:** The authors thank the German research foundation (DFG) for financial support within the project LE817 / 5-1.

P35 (EI0137)

Application of A Hybrid Model to Continuous Flow Pump Investigation

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Objectives: Continuous flow pumps (CFP) are widely used and differ from each other in types, connection and performance. Circulatory and ventricular conditions too play a role in pump performance. The number of variables involved makes modeling an appealing tool to study pump performance in different patient's conditions and to support to clinical decision. This work aims at developing a circulatory model merging a computational model (CM) with a CFP working in its own environment. In this way it is possible to investigate the CFP effects on variables calculated by the computational model but influenced by the pump. **Methods:** The lumped parameter CM consists of left and right hearts, systemic, pulmonary and coronary circulation. The CFP is represented by an electrical model realised using operational amplifiers. The pump speed and the slope of the pressure-flow characteristics can be controlled to simulate different pump types. Pump performance was analysed considering circulatory (Cardiac Output-CO, left atrial pressure-LAP, aortic pressure-AOP) and ventricular variables (ESV and EDV) against ranges of left ventricular E_{maxl} (0.5–2.5–3.5 mm Hg· cm^{-3}) and stiffness (30–60 cm^3 ·mm Hg^{-1}). All experiments were conducted comparing the selected variables before and after pump activation. The pump was connected between left atrium and aorta. **Results:** Chosing maximum pump speed and $E_{max} = 3.5$ mm Hg ·cm^{-3}, CO, LAP and AOP variations are remarkable: 45%, –300% and 73%, respectively. The pump flow shape is influenced by the pressure variations across the pump head. Different ventricular and circulatory conditions produce remarkably different effects and it is possible to identify dangerous situations (suction). **Conclusions:** This work demonstrates the possibility to merge a CM with a device working in a different environment. The model provides a platform to produce stable and repeatable circulatory conditions. The CM will be connected to a real device using the technology developed in the frame of EU SensoART project.

P36 (EI0068)

A Novel Implantable Sensor to Monitor Both Apical Rotation and Cardiac Phases

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Objectives: The magnitude and timing of left ventricular (LV) twist with respect to cardiac phases are essential to detect systolic and diastolic dysfunction, as recently shown by advanced imaging techniques. No implantable sensors are currently available to provide that phasic analysis of LV rotation over the cardiac cycle. We developed and evaluated in a sheep model an innovative implantable gyroscopic sensor for the continuous endocardial monitoring of both the amount and timing of cardiac rotation. **Methods:** In a sheep, a tip catheter gyroscopic sensor was inserted in the endocardium of the right ventricle apex. The detected signal (EndoTwist) was continuously recorded along with ECG, LV pressure (LVP) and its first derivate (LVdP/dt). EndoTwist was processed in order to obtain both cardiac rotation parameters (twist rate ω, apical rotation angle θ) and mechanical heart vibrations (MHVs) used to identify systole and diastole. **Results:** The detected EndoTwist signal clearly showed both a low-frequency component relating to cardiac rotation (ω, θ) and a high frequency component relating to MHVs. Identification of systole and diastole from MHVs was confirmed by comparison with LVdP/dt which was previously used to define the timing of the cardiac cycle. **Conclusions:** The new implantable sensor permits detection of cardiac twist dynamics (ω, θ) with respect to the entire cardiac cycle by means of MHVs recognition. This information, if confirmed in larger studies, has promising clinical implication for the monitoring of cardiac function in heart failure patients.

P37 (EI0060)

The Establishment of The Quantitative Evaluation Standard for The Anatomical Compatibility of The Ventricular Assist Device

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Objective: The body surface area (BSA) is currently used as a standard to determine the anatomical compatibility for patients with a ventricular assist device (VAD). However, it is difficult to accurately evaluate the anatomical compatibility for the patients when the patient's BSA measurement is close to the standard value of BSA. The purpose of this study is to establish a new quantitative standard that is more accurate than the currently used BSA standard. **Method:** BSA values of subjects were calculated using the height and the weight. The three-dimensional (3D) models of the chest and abdomen were constructed on the computer by means of the image processing software using the computerized tomography (CT) images of the chest and abdomen for subjects. The volumes of the chest and abdomen (VCA) were calculated by means of the 3D models. In this study, the relationship between the BSA and the VCA was examined for subjects who have same BSA value. **Result:** As an example, the resulting BSA and VCA measurements for two subjects showed that even though the BSA measurement was 1.6 for both subjects, the VCA measurements of 61 and 53 revealed a large difference. **Conclusion**: The VCA was evaluated as a new quantitative standard. The results of this study suggest that VCA give detailed information to us for the standard to evaluate anatomical compatibility than BSA.

P38 (EI0084)

A New Integrated Hybrid Cardiovascular Simulator as A Smart Tool for VAD Development, Design, and Research

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Objectives: Why not simulate VAD-Heart interaction easier and better? Pure physical cardiovascular simulators have many limitations, are not flexible, and do not reproduce precisely many parts of circulatory system. Our main goal was to develop a VAD-Heart Simulation Platform (VHSP)—hybrid multipurpose tool enabling mechanical assist devices interfacing connection with a virtual (numerical model) circulatory system (VCS). **Methods:** Four hydro-numerical impedance transformers (TR) integrated into one assembly were coupled with a VCS working in real-time. Each TR may be configured independently as an input or output channel connecting inflow or outflow cannula of VAD to the selected point of VCS. We can obtain a parallel or serial LVAD/RVAD assistance using two TRs. Four TRs enable BVAD application. Adding an artificial valve as e.g. mitral or aortic one, we extend a field of VHSP applications to e.g. valve's investigations. **Results and Discussion:** VCS is seen from the VAD point of view as a physical model, but VCS is still numerical, so changing a computer software we can tailor a structure of VCS to the specific requirements. Change of any VCS parameter is only a few mouse clicks. Thanks to the TRs we can connect to VCS different pulsatile, nonpulsatile or centrifugal assist devices. Some experimental courses illustrating VCS performance are also included. **Conclusions:** Presented VHSP is a result of longstanding Polish-Italian group cooperation. Some applications like parallel LVAD/RVAD/BVAD assistance are available. The next one will be released in near future. We are planning to enrich VCS introducing some physiological mechanisms like e.g. interventricular interaction, baroreflex feedbacks and cardio-pulmonary interaction. Potential VHSP applications are new VAD development as well as medical staff training. **Acknowledgements:** The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement num. 248763 (SensorART Project).

P39 (EI0231)

Cfd-Aided Design Optimization of a Centrifugal Blood Separating Device

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Objectives: The purpose of this work is to define innovative strategies, based on advanced computational methods, to optimize the geometry and the functioning of a centrifugal hemoconcentrator. When autologous blood is retrieved from drainage during or after surgical operations, the device allows to apply a centrifugal force field on blood in order to separate red blood cells (RBC) from plasma and from waste products. The separated portion of blood is then reinfused into the patient, thus avoiding the risks associated to allogenic blood transfusions. **Methods:** Geometrical and functional optimization of a blood separator was performed by means of computational fluid dynamics (CFD) simulations. The transient process of apheresis was simulated with a multiphase

mixture model in which two phases were defined: the granular RBC phase and the primary plasmatic phase. Blood was modeled as a Newtonian fluid, density values of 1030 Kg/m^3 and 1095 kg/m^3 were used respectively for plasma and RBC. A 33 ml/min flow was assigned at the inlet with a erythrocyte volume fraction of 0.1, and a centrifugal acceleration of 900 g was used. The maximum packing limit for the particulate phase was set to 0.7 in order to account for limited RBC deformability. Different design solutions were tested. **Results:** CFD results in terms of RBC volume fraction and velocity fields within the device were compared among different geometries. This allowed assessing the hemoconcentrator efficiency working with a low hematocrit blood (Hct = 10%) as inflow. Furthermore, the device performances were evaluated by varying the inlet flow rate, the rotational speed and the shape of the outlet conduits. **Conclusions:** On the basis of CFD simulations results, new and improved geometrical configurations were proposed for the hemoconcentrator, in order to avoid vortex development and to increase the separation rate up to a 60% hematocrit concentrated blood at the outlet.

P40 (EI0255)

In Vivo Evaluation of a Wearable Pneumatic Total Artificial Heart System with a Compact Drive Unit

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Objectives: We have been developing a wearable pneumatic total artificial heart (PTAH) system with compact wearable pneumatic drive (WPD) unit for bridge to transplant. This paper reports current state of development including the results of the first acute animal experiment in PTAH system. **Methods:** The PTAH system consists of left and right diaphragm-type blood pumps and two WPD units. Two blood pumps were designed to fit anatomy, and these had housings made of polyurethane resin and stainless steel. 25 mm ID and 23 mm ID Bicarbon valves were mounted in the inlet and outlet ports, respectively. The sizes of the left and right pumps are $95 \times 68 \times 49$ mm and $104 \times 74 \times 44$ mm. The stroke volume of the left and right pumps are 85 and 94 ml. The WPD unit consists of a brushless DC motor, a crankshaft, a cylinder-piston, noncircular gears, and air pressure regulation valves. Driving air pressure is generated by the cylinder-piston. The noncircular gears generate the fixed systolic ratio. The size and weight of the WPD unit are $20 \times 8.5 \times 20$ cm and 1.8 kg, respectively. The left and right pumps were implanted in a calf weighing 98 kg. Two WPD units with fixed systolic ratio of 35 and 44% were connected to left and right pumps with 2 m drive lines, respectively, and fundamental performance of PTAH system was evaluated in acute animal experiment. **Results:** The cardiac output ranged between 3.9 and 5.4 L/min at the mean aortic pressures of 108–115 mm Hg at pump rates of 60 to 100 bpm. The average electric power consumption in each WPD unit varied from 5.3 to 17.6 W according to the beating rate, and the efficiency was estimated to be 2–9%. **Conclusions:** These results indicated that our PTAH system consisting of blood pumps and WPD units have sufficient performance for total cardiac replacement.

P41 (EI0328)

Numerical Method of Flow Stagnation Areas Estimation Under Steady State Conditions in Pneumatic VAD

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Objectives: A numerical flow analysis of pneumatic VAD, POLVAD-MEV type in steady state conditions was performed. New numerical method of flow stagnation areas estimation in VAD was proposed. Results were compared with fibrin deposition detected in POLVAD-MEV pump after clinical applications (46 VADs used in four clinical center in Poland). **Methods:** Flow simulations for two opposite operational states (end-diastole and end-systole) were simulated. In order to fulfill code requirements in steady state, both discs were open. For diastole, the inlet disc was fully open whereas the outlet one was almost closed. For systole, it was vice versa. The non-Newtonian blood model based on the Power Law was applied in numerical simulations, utilizing ANSYS CFX v.13 code. Streamlines and flow stagnations areas in the blood chamber had been analyzed. 46 VADs were collected from cardiac centers after heart support. The VAD blood chamber were divided into sections and evaluated regarding biological deposition detection and analyzing. **Results:** The flow stagnation areas estimated numerically have good correlation with localization of fibrin deposition detected in pumps collected after clinical application. **Conclusions:** The numerical study shows that steady state simulations are useful in the design of internal VAD geometry as they need not extreme computational efforts. Clinically collected material confirmed good correlation of numerical analyses, applied for new ventricular assist devices designing in the Artificial Heart Laboratory.

P42 (EI0344)

Development of Durable Rotary Blood Pump Systems Using Hydrodynamic Bearing Technology

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Objectives: A hydrodynamic bearing system uses the fluid film pressure to keep the noncontacting rotation of the impeller of a rotary pump. This is one of the key technologies to develop a blood pump system with superior durability and antithrombogenicity. Our research team is currently working on development of two different blood pump sytems with hydrodynamic bearing systems. **Methods:** (i) Cardiopulmonary support system with a centrifugal pump The extracorporeal centrifugal blood pump has the dual hydrodynamic bearing systems in the single impeller. The prototype blood pump system is composed of a disposable centrifugal pump head with the compact driver containing a DC brushless motor. The dimensions of the pump with the driver are 75 mm(diameter) × 135 mm, weighing 500 grams. The priming volume of the pump is 18 mL. (ii) Implantable left ventricular assist device (LVAD) with an axial flow pump. The first hydrodynamically levitated axial flow pump was developed in this project. The pump has the dimension of 29 mm in diameter and 75 mm in length. The weight of the pump is approximately 150 grams. The impeller rotor has a bore in the center that form a blood film with the shaft connecting the diffuser and the inlet flow devider. The power consumption of the prototype is about 5 watts at typical working condition as an LVAD. **Results:** Both systems were evaluated through a series of chronic animal experiments up to 1 month for the cardiopulmonary support and 3 months for the implantable LVAD. The results of the animal experiments demonstrated a superior blood compatibility of the pumps. **Conclusions:** We have succeeded in development of blood pump systems for long-term use applications including a cardiopulmonary support system and an implantable LVAD system. This research program is supported by New Energy Development Organization (NEDO) of Japan.

P43 (EI0288)

Development of a Mock Circulation System for Endurance Test of Ventricular Assist Devices

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Objectives: We have been developing a mock circulation system for endurance test of ventricular assist devices that can simulate a variety of physiological circulatory conditions. In this study, we compared several inflow and outflow valves for generating pressure and flow waveforms of the systemic circulation on the basis of waveform analysis. **Methods:** The mock circulatory loop for endurance test consisted of a pulsatile pump with two closed-chambers (left ventricle and aortic compliance), a control valve (peripheral resistance) and a reservoir (left atrium). Evaluated valves (mechanical prosthetic valves, umbrella and duckbill valves) were mounted in the inlet and outlet of the left ventricle. The pump was driven at 70 bpm, and the aortic pressure (AoP) was set at the 120/80 (100) mm Hg. Left ventricular pressure (LVP) and AoP were measured for 1 minute in each valve. Each waveform was evaluated by the spike component extracted with median filter processing, the vibrational component extracted with frequency analysis (FFT) and the slope of each pressure waveform calculated with differential processing. **Results:** Examples of obtained evaluation indices were shown as follows: The peak-to-peak of filtered LVP (spike components) and integrated values of power spectrum in spike elements by FFT (vibrational components) were 345.8 mm Hg and 518.1 mm Hg^2 in mechanical valves, 218.8 mm Hg and 522.1 mm Hg^2 in umbrella valves and 162.7 mm Hg and 142.4 mm Hg^2 in duckbill valves, respectively. Thus, the duckbill valve showed the most favorable characteristics in which pressure waveforms were close to the nature systemic circulation. **Conclusions:** The endurance test circuit under development was able to generate pressure waveforms with low spike and vibrational components by using duckbill valves. It was suggested that our system has a potential to evaluate various characteristics of prosthetic valves by the simple wave pattern analysis.

P44 (EI0403)

Switching from Vv To Va Bypass In A Patient With Severe Ards: Insight From A Case Report

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Objective: To describe clinical consequences while switching from VV to VA bypass in a patient with severe ARDS. To suggest the use of combined VV and VA ECMO support or a VA support with central cannulation in ARDS patients with concomitant cardiac failure. **Methods:** A 62 years old mechani-

cally ventilated patient with hypoxemic respiratory failure due to Influenza A (H1N1) virus, septic shock, multiorgan failure, and unresponsive to rescue therapies, was connected to a VV femoro-femoral ECMO circuit (blood flow 3.4 l/min, gas flow 6 l/min). In spite of an improvement in oxygenation, shock worsened with signs of tissue hypoxia and lactic acidosis. After percutaneous incannulation of the femoral artery the bypass was converted from VV to VA. Following bypass conversion, despite an ECMO blood flow up to 6 l/min, we observed severe desaturation, increase heart rate, decrease arterial blood pressure and cyanosis of the upper body. The patient was therefore connected to a modified extracorporeal circuit consistent in one venous drainage and two reintroductions, one venous (3.4 l/min) and one arterial (2.5 l/min) with two separated ECMO circuits. Vital parameters rapidly improved while arterial lactate decreased and global perfusion ameliorated. To understand the events observed during the switch from VV to VA bypass we tested different combination of venous and arterial blood flow. **Results:** Decrease of VV blood flow while increasing VA blood flow was associated with a severe reduction in mixed venous oxygen saturation and PaO_2, probably caused by a high degree of recirculation of highly saturated blood in the lower body. Cardiac output slightly increased despite increasing arterial ECMO support, likely due to the worsen oxygenation of the upper body. **Conclusions:** In a patient with severe respiratory failure and impaired cardiac function, a peripheral femoral VA bypass may not be appropriate to restore adequate physiological parameters.

P45 (EI0076)

Liposome-Encapsulated Hemoglobin Reduced Cerebral Infarct Volume in Rodent Transient Focal Ischemia

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Objectives: Liposome-encapsulated hemoglobin (LEH, Terumo, Tokyo) is reportedly protective in cerebral ischemia and reperfusion. Search for the optimal oxygen affinity revealed that LEH with high affinity (hLEH, P_{50} = 10 mm Hg) was more effective than LEH with low affinity (lLEH, P_{50} = 40 mm Hg). In this study, we explored LEH with mid-level affinity (mLEH, P_{50} = 17 mm Hg). **Methods:** mLEH (2 mL/kg, n = 8) or vehicle (saline, n = 7) was intravenously infused 5 minutes after thread occlusion of the middle cerebral artery in the rat. The thread was removed after 2 hours for reperfusion and the infarction area was assessed by TTC staining 24 hours later, with periodic neurological function and cortical blood flow monitoring. **Results:** While relevant cortical blood flow and neurological function tended to be preserved in mLEH-treated animals, infarct volume in the cortex (98 vs 207 mm^3, $P < 0.01$) and striatum (92 vs 164 mm^3, $P < 0.01$) and edema formation (106% vs 130%, $P < 0.001$) were significantly better suppressed in mLEH-treated rats than in vehicle-receiving control animals. **Conclusions:** mLEH reduced edema formation and infarct volume after cerebral ischemia and reperfusion in the rat. This protective effect appears to be no less potent than that of hLEH or lLEH.

P46 (EI0003)

Variation of Local Elasticity along The Length of The Aorta as Observed by A Scanning Haptic Microscope (SHM)

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Objective: Variations in microscopic elastic structures along the entire length of beagle aorta were evaluated using a scanning haptic microscope (SHM). **Methods and Results:** The total aorta from the aortic arch to the abdominal aorta was divided into 6 approximately equal segments. After embedding into agar, each segment was cut in circumferential cross-section to obtain disk-like samples with flat surfaces (thickness, approximately 1 mm). The surface elasticity and topography of the samples under nonload and zero-stress conditions were simultaneously measured along essentially the entire wall thickness by SHM, using a probe with a diameter of 5 µm and a spatial resolution of 2 µm at a rate of 0.3 s per point. Elasticity in the wall was the highest at the luminal surface side and decreased gradually toward the adventitial side. The tendency was similar to that of the change in the elastin fiber content. In the evaluation at the mid portion of each segment, the highest elasticity (40.8 [3.5] kPa) was identified at the thoracic section of the aorta that had the highest concentration of elastic fibers. **Conclusions:** Under nonload and zero-stress conditions, the elasticity of the aorta was determined by the elastic fiber content.

P47 (EI0169)

Wearable Artificial Kidney: Maintaining Body Homeostasis through Constant Hemofiltration

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Objectives: Steady euvolemia in ESRD (end stage renal disease) patients is almost impossible to maintain with currently available treatment options. Inability to maintain constant dry weight causes discomfort, uncontrollable hypertension and intra- and post- dialytic hypotension that compromise solute removal. We have designed a wearable artificial kidney, an ambulatory ultrafilter that will maintain invariant dry weight and, when used in conjunction with twice-weekly in-clinic dialysis, can provide adequate solute removal. **Methods:** The microsieve is constituted of silicon-rich silicon nitride (Si_xN_4, x > 3), a hydrophobic non-biocompatible material, which has recently been micro and nanopatterned to form channels and filtering surfaces that may become valuable components of artificial organs. This material has been rendered hydrophilic and potentially hemocompatible through two different mechanisms: attachment of zwitterionic moieties and deposition of Ti metal followed by oxidation and annealing to TiO_2-anatase. Zwitterionic poly(sulfobetaine acrylamide) (SBMAA) brushes were grafted onto perforated semiconductor microsieves by Atom Transfer Radical Polymerization (ATRP) and, were then, studied in human blood filtration experiments. ATRP initiators were immobilized onto Si_xN_4 through stable Si-C linkages via 3 consecutive reactions. Zwitterionic polymer brushes of SBMAA were grown (thickness ~ 30 nm) from these initiator-coated surfaces and the polymer-coated surfaces were characterized in detail by static water contact angle measurements, X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM). **Results:** Both the Zwitterionic and Ti-O treatments showed low contact angles, indicating the surfaces have been made hydrophilic. Furthermore, when exposed to blood on a microfluidic blood plasma separation module (BPSM), a significant decrease was observed in erythrocyte adhesion, platelet aggregation, and protein adsorption. As a result, filtration rate through the microsieve increased by 90–180%. **Conclusions:** The device is thus capable of constantly extracting 1 ml/min, about 10 kg of ultrafiltrate per week and is designed for inspection and servicing in each dialysis session.

P48 (EI0257)

Tissue Engineering of Magnesium Stabilized, Vascularized, Autologous Gastric Tissue for Cardiac Muscle Replacement

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Objectives: Surgical replacement of diseased cardiac muscle often is the therapy of choice. At this biological patches outmatch synthetic grafts. We showed physiologic in vivo remodeling following autologous transplantation of a vascularized segment of small intestine. Nevertheless application of such tissue as myocardial patch in the left high pressure area of the heart is hardly possible due to its mechanical instability. Hence it was to be examined in this trial if stabilizing of the biological patch by a degradable magnesium scaffold would impair the remodeling and healing process. **Methods:** An area of 2 cm of diameter in the left ventricular myocardium of Minipigs (n = 9) has been replaced by autologous, muscular segments of the stomach including native arterial and venous vessels. This patch was epicardially fixed by a specially designed magnesium scaffold. The grafts were explanted following 1, 3, and 6 months after the surgical procedure and were assessed histologically. Possible enrichment of magnesium and its degradation products was examined chemically. Degradation of the magnesium scaffold was tested with microcomputer tomography. **Results:** All animals survived the surgical procedure. Metallic debris was found in the gastric patch and the surrounding myocardium. Increased enrichment of magnesium or its degradation products was not observed in the kidneys, liver, skeletal muscles, and myocardium nor in the bones of the examined pigs. There was no evidence of cytotoxicity of the implanted magnesium. A sterile granulomatous inflammation and a very good capillarization were found up to 6 months after implantation. **Conclusions:** The autologous vascularization in our approach is a prerequisite for the in vivo remodeling. The use of degradable magnesium scaffolds to stabilize vascularized gastric tissue allows for application of this initial fragile biological graft in the cardiovascular high pressure area. This trial shows the biological compatibility of degradable magnesium as epicardial scaffolds.

Functional Biomaterials

P49 (EI0414)

A Highly Sensitive Biosensor For Detection of Tnf-Cytokine To Predict The Biocompatibility of Transplanted Organs

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Objectives: Heart failure (HF) is a condition where the heart fails in its duties of circulating blood through the lungs and back out to the tissues. Diagnosis of acute rejection is a complex and persistent problem in heart and ventricular assisted device (VAD) transplantation. To address this problem, measuring specific biomarkers (e.g. TNF-α, IL-1 and IL-10) can produce immediate information about the first signs of inflammation. These biomarkers are usually present within the inflamed organ with high levels and plays a major role in coordinating mechanisms which command inflammatory response. **Methods:** Fabrication of flexible gold microelectrodes based on polyimide for direct detection of TNF-α required no labeling. Electrochemical impedance spectroscopy (EIS) of the heterostructures, Au/covalently bonded antibodies/buffed medium, was utilized for monitoring the specific antibody-antigen interaction. Experimental parameters affecting antibody immobilization and the sensing of TNF-α were investigated in detail and optimized. **Results:** Nyquist plots provide high sensitivity and selectivity for TNF-α versus the antigen IL-1 and IL-10 under the optimized experimental conditions. A linear response was obtained in the concentration range 0,02 pM to 2 pM. The developed biosensor showed a sensitivity of 1,107 M^{-1} and a limit of detection of 0,02pM. Small responses were observed when IL-1 and IL-10 where measured, showing the selectivity of the biosensor. The reliability and applicability of the developed biosensor was also demonstrated. **Conclusions:** The aim of this work was to manufacture a flexible biosensor for detection of TNF-α without any labeling, using electrochemical impedance measurement rather than the traditional techniques. The developed biosensor can be potentially applied for point–of-care applications.

P50 (EI0037)

Hemoadsorption of High-Mobility Group Box 1 in Swine Acute Liver Failure Model

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Background: We investigated if adsorption of high-mobility group box 1 (HMGB-1) is feasible and beneficial for treatment of acute liver failure (ALF) in swine model. **Materials and methods:** i) Establishment of animal model. Adult male swine were injected with D-galactosamine (0, 0.2, 0.6, or 1.0 g/kg) to induce ALF. The serum parameters and histological examination in the liver were assessed. Survival was observed for 7 days. ii) In vitro absorption study. A multi-cytokine adsorbing column (CYT-860, Toray Inc. Tokyo, Japan) was newly established. The plasma samples containing HMGB-1 were incubated with the fibers of column for 2 hours. (iii) Extracorporeal perfusion study. The swine model of 0.6 g/kg was subjected to extracorporeal direct hemoperfusion study. Perfusion was performed for 4 hours using the column and the HMGB-1 levels at the inlet and outlet of the column were determined. Heptic enzymes were determined at 36 hours after ALF induction. Survival was observed for 7 days. **Results:** i) The levels of TB, AST, LDH, and HMGB-1 showed significant elevations in the groups of 0.6 and 1.0 g/kg. Survival study showed that the outcome was dose dependent. Histological examination of the liver showed hemorrhage and necrosis in the groups of 0.6 and 1.0 g/kg. ii) In vitro study showed that the fibers adsorbed 94.3 3.1 % of HMGB1. iii) The level of HMGB-1 was markedly suppressed in the outlet compared to the inlet of column during the perfusion with CYT-860. The levels of AST and LDH were markedly suppressed in the group with CYT-860 36 hours after ALF induction ($p = 0.06$ in AST, $P < 0.05$ in LDH). There was a tendency that the survival was improved in the group with CYT-860 column compared to control column ($p = 0.07$). **Conclusion:** The newly established cytokine-adsorbing column reduced the serum HMGB-1 level and may be beneficial for ALF treatment.

P52 (EI0325)

Micropatterning of Bioactive Glass Nanoparticles on Chitosan Membranes for Spatial Controlled Biomineralization

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Objectives: Chitosan membranes were patterned with bioactive glass nanoparticles (BG-NPs) capable of bone regeneration by a Microcontact Printing technique, in order to spatially control biomineralization and also cell adhesion and proliferation. **Methods:** After "inking" an elastomeric stamp in BG-NPs, it was pressed against the chitosan substrate and then lifted off, in order to transfer a perfectly defined bioactive micropattern. The mineralization of the bioactive glass patterns was induced *in vitro* by soaking the samples in simulated body fluid (SBF) over several time points up to 7 days. The interaction between cells and patterned membranes surface was evaluated, by seeding L929 fibroblasts cells over 1, 3 and 7 days on their surface. **Results and Discussion:** The induction of confined mineralization was confirmed by FTIR, EDX and SEM. Cell adhesion and proliferation were studied by means of scanning electron microscopy (SEM). The results showed that the produced patterned membranes succeeded in controlling mineralization, cell adhesion and proliferation. MTS assay confirmed that cellular viability increased with time of culture. The developed BG-NPs micropatterned chitosan membranes can be applied in *In situ* tissue regeneration. **Conclusions:** The produced membranes proved to be a suitable substrate for cell growth, being the BG-NPs a highly reactive surface able to bond with living cells. Total control of cell attachment and spatial biomineralization was achieved through micropatterning of BG-NPs on chitosan membranes.

P53 (EI0317)

New Method Based on Rt-Qpcr for Biocompatibility Testing of Dialysis Filter Devices

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Objectives: Objective of this work is to provide a new and more sensitive method based on RT-qPCR (quantitative polymerase chain reaction) for biocompatibility testing in order to facilitate further improvements of dialysis filter devices. Need for improved biocompatibility is given by the clinical observation that dialysis patients have increased risk of cardiovascular disease that is suspected to be influenced by the foreign surface contact. Although there exist a couple of methods to measure biocompatibility, these methods are not sensitive enough to differentiate biocompatibility of modern filter devices and consequently these methods are not suited to guide future developments. **Methods:** The method is a two-step *in vitro* process. The first part is the exposure of human blood in parallel to two dialysis filter devices. The second part is the quantitative analysis of the activation level of the leukocyte cell population. The activation level is quantified by the amount of mRNA of a specified set of inflammatory markers by real-time quantitative PCR. Additional information is obtained by FACS analysis of surface marker proteins. **Results:** RT-qPCR analysis of inflammatory markers in human blood showed that exposure of blood to different filter devices resulted in different leukocyte activation levels. Particularly early inflammatory markers like TNF-a and IL-1ß revealed statistically significant differences between filter devices. Though physical and chemical analysis of the filter materials showed differences in filter materials, no single parameter could be correlated to increased leukocyte activation. **Conclusions:** A new method based on RT-qPCR could be established for biocompatibility testing of modern dialysis filter devices. The method allows *in vitro* characterization of inflammatory processes that are caused by foreign surface contact and are suspected to be clinically relevant for cardiovascular complications. Moreover, the method showed differences in leukocyte activation between commercial dialysis filters and is therefore suited to guide further improvements in biocompatibility.

P54 (EI0087)

Functionalized Polypropylene Mesh for Incisional Hernia Regeneration

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Objectives: The aim of this study was to develop functionalized scaffold for incisional hernia regeneration. New composite scaffolds, based on polypropylene chirurgical mesh (PP), poly-e-caprolactone (PCL) nanofibres, and thrombocyte-rich solution (TRS) have been prepared and tested in *in vitro* study

using 3T3 fibroblasts. **Methods:** Four different samples have been prepared: PP, PP covered with PCL nanofibres (PP+PCL), as well as PP and PP+PCL functionalized with immobilized thrombocytes (PP+TRS and PP+PCL+TRS, respectively). Nanofibres were prepared by the electrospinning method from the chlorophorm/ethanol solution. To achieve thrombocyte immobilization, PCL nanofibres were immersed in a thrombocyte-rich solution for 2 hours. 1×10^3 3T3 fibroblast were seeded onto each scaffold and cultured for 14 days. Cell proliferation and viability were evaluated on the day 1, 3, 7, 10, and 14 by MTT assay and live/dead stainig (BCECF- AM and Propidium iodide) with subsequent confocal microscopy visualization. **Results:** Biocompatibility of functionalized surgical mesh, cell proliferation and viability was determined using MTT test and confocal microscopy. The regenerative potential of thrombocytes was based on release of growth factors that occurs when thrombocytes rupture. MTT test demonstrates significant increase in cell number on scaffold covered with PCL and functionalized with immobilized thrombocytes. These results correlated well with live/dead staining. Viability of cells 14 days after seeding was 95 %. **Conclusions:** Polypropylene surgical mesh was covered with PCL nanofibre layer, functionalized with immobilized thrombocytes and seeded with 3T3 fibroblasts. Cells proliferated well on the functionalized scaffold during a 14 day experiment. Very good biocompatible properties of this scaffold were observed. This material will be tested and has a good potential to be clinically used. Acknowledgements: Grant Agency of Czech Republic (grant No. P304/10/1307), The Grant Agency of the Charles University (grant No. 97110, 164010).

P55 (EI0059)

Re-Endothelialization of a Biological Vascularized Matrix (Biovam) For the Generation of 3D Artificial Tissues

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Objectives: Tissue engineering is a promising technique for reconstruction of failing organs. Based on its size the supply of cells with nutrients and oxygen in constructs requires an in vitro and/or in vivo vascularization e.g. a biological vascularized matrix (BioVaM). Here we report the generation of a vascularized matrix for 3D artificial tissues. **Methods:** An established decellularization process utilizing Na-deoxycholate and SDS was used to generate a matrix with preserved pedicles derived from porcine small intestine. The morphology of the decellularized vessel bed of the matrix was characterized by ink injection and immunostaining. The maximal injection volume of the vessel bed lumen was determined. The matrix was recellularized with lentiviral stable transfected RHE (=rat heart derived endothelial cell line) cells. GFP labeled cells were infused into the venous and RFP labeled cells were infused into the arterial vessel bed. The reseeded matrix was cultivated for 2 weeks under static and/or perfused conditions. After cultivation the whole construct and cryosections were analyzed via fluorescent microscopy. **Results:** Ink injection into the decellularized matrix revealed fine and distinct structures for the arterial vessel bed, whereas the venous vessel bed showed broad and leaky ink distribution. A maximal injection volume was determined. With the adjusted injection volume reendothelialization of arterial and venous vessel bed was achieved after 14 days of static cultivation of the matrix. Perfusion of the matrix was beneficial for the repopulation of big vessel structures. **Conclusions:** The arterial vessel bed is well preserved after decellularization; the venous vessel bed is more susceptible to damage due to its native structure of a thin muscular layer. Reendothelialization of the matrix was already achieved under static conditions; however further perfusion might improve functional vessel formation. Perfusable, endothelialized constructs may aid in solving the problem of nourishing cells inside 3D tissue-engineered constructs.

P56 (EI0268)

Innovative 3D Biotextiles for Potential Bone Tissue Engineering Applications

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Objectives: Bone tissue engineering (TE) represents a specialised niche within the biomedical field to which textile technologies can markedly contribute. Textile technologies are considered as potential routes for the production of scaffolds for TE applications, as they present superior control over design and reproducibility. This work aims at developing novel 3D textile structures based on different polymeric materials and to engineer their surfaces in order to promote and control cell adhesion and proliferation. **Methods:** Natural and synthetic polymers such as silk, polybutylene succinate (PBS) and poly(ethylene therephtalate) (PET) were selected to be extruded into multifilament yarns and processed into different structures such as Jersey, Rib and Piqué and 3D spacer. Different surface modifications were performed (acid/alkaline treatment, UV radiation and plasma) for increasing cell adhesion and proliferation. The immobilization of different proteins on the surface of modified materials was also performed. All textile constructs were characterized in terms of porosity, morphology and mechanical properties by μ-CT, SEM and DMA. The effectiveness of the surface modifications was assessed by FTIR, XPS and contact angle measurements. **Results:** The obtained constructs present very reproducible intra-architectural scaffold geometry with high surface area and exhibiting a wide range of porosities. By the above mentioned techniques it was possible to validate the effectiveness of the proposed treatments in modifying the surface of the materials. In addition, Bovine Serum Albumin was successfully immobilized on the obtained surfaces. Cell adhesion and proliferation studies validated the developed constructs for the proposed application. **Conclusions:** The proposed textile methodologies made possible the development of highly reproducible constructs featuring a wide range of porosities and surface areas. The effective modification and immobilization of biomolecules on the surface of the biotextiles has potential for modulating cell response and optimize overall biological performance.

P57 (EI0161)

Polymer Surfaces Coated with Hydrogel to Improve Blood Compatibility

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Objectives: The aim of presented research was to develop a method for manufacturing hemocompatible coatings for blood-contacting devices. We present a simple method for fabrication of hydrogel coatings for cardiovascular devices. Polyvinylpyrrolidone (PVP) was chosen as a hydrophilic polymer to produce hydrogel network due to its highly biocompatibility and wide applications in medicine. **Methods:** Hydrogel coatings of polyurethane (in a form of discs) were fabricated in a two-step method. First, the PU discs were immersed in a solution containing given amounts of crosslinking agent (EGDMA) and cumene hydroperoxide for 15 minutes at 25°C. After that time, samples were placed in a water solution containing given amounts of PVP, $FeCl_2$ and ascorbic acid for 15 minutes at 25°C. Polymer discs were then washed and dried. Blood-biomaterial interactions were evaluated using a platelet analyzer (Impact-R, DiaMed). A given volume of a whole-blood sample was dropped onto the characterized surfaces and shear stress was applied to simulate arterial flow conditions. The platelet consumption was calculated as a difference between the initial number of platelets present in blood sample and the number of platelets after the test. **Results:** Presented method is based on free-radical macromolecular polymerization. Cumene hydroperoxide is a source of radicals produced in the redox reaction with Fe^{2+} ions. Macroradicals recombination leads to PU-PVP grafting, PVP crosslinking and hydrogel formation. The results showed that the platelet consumption decreased from 56% (for unmodified PU) to 10% (for PU grafted with PVP). **Conclusions:** Polyurethane grafted with polyvinylopyrrolidone seems to be promising material for cardiovascular applications. Hydrogel coating greatly reduced the level of platelet adhesion and activation.

P58 (EI0243)

Permanent Central Venous Catheter with A Lock Balloon

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Objectives: Permanent vascular access is essential for hemodialysis, parenteral nutrition and drug administration. Mostly a central venous catheter (CVC) is used. However, this use poses a problem: infection. The cause is the intraluminal space, which acts as a bioreactor during the time when the CVC is idle. To prevent this, a bactericidal liquid, called a lock solution, is injected into the intraluminal space. For fluid mechanical reasons, it is not possible to completely fill the intraluminal space without injecting the lock solution into the bloodstream. The proposed lock balloon fills the intraluminal space and makes a lock solution redundant. **Methods:** A standard single lumen central venous catheter of 1.4 mm inner and 2.1 mm outer diameter was chosen to be equipped with a lock balloon. The latter was fabricated from 25 micrometer polyurethane membrane. The membrane was inserted into the catheter and glued in. A bi-stable actuator was designed and attached to the lock balloon. The lock balloon is filled with 0.3 milliliter air. **Results:** During infusion the lock balloon is collapsed and the lumen is free for the passage of the infused liquid. However, the cross section is reduced and the resistance is doubled. After infusion the lock balloon can be inflated again and then completely fills the intraluminal catheter space. The bi-stable actuator permits to activate and deactivate the lock balloon like a switch. **Conclusions:** The concept appears valid and further investigations will deal with miniaturization of the lock balloon and animal experiments. The animal experiments are designed to model the routine of CVC use. Conventional CVCs will be compared to lock balloon CVCs. Blood cultures after catheter use will be used as a control.

P59 (EI0241)

A Catheter Model for The Evaluation of Anti-Biofilm Agents in Rats

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Objectives: The colonization of indwelling catheters by bacteria and the formation of biofilms is a frequent problem in today's clinical setting. These infections, mostly caused by coagulase negative staphylococci, are among the most common nosocomial infections and may even cause the sepsis syndrome. The biofilms are often highly resistant to conventional antibiotics. In this study we evaluate the effect of the antibiotic, e.g. daptomycin whose therapeutic mechanism is supposed to also affect bacterial biofilms. **Methods:** A previously published rat model was modified using a peripheral venous catheter for human use that was adapted for the application in rats. For this study the Teflon tip of the catheter was replaced by a polyurethane tube, to soften and to prolong the line. The system was sterilized using formalin gas and inserted in the right vena jugularis externa. The proximal tip of the catheter was subcutaneously tunneled and passed outwards between the scapulae. To secure the position of the catheter the adjacent plastic wings were sued onto the adjacent tissue. Then the lumen was inoculated with *S. epidermidis* and allowed to indwell within the catheter for 7 days. Afterwards antibiotics were infused in different rats according to their standard instructions for human use. Saline was used as negative control. The rats were sacrificed and the catheters explanted. After embedding in methacrylate and sectioning, fluorescence-in-situ-hybridisation technique (FISH) was used for the quantification of microorganisms and bacterial activity. **Results:** The system was well suited for the respective experiments and was well tolerated by the animals. Biofilms were successfully grown in the catheters and could be visualized and quantified using the FISH technique. **Conclusions:** This catheter model allows the in-situ-analysis of different anti-biofilm strategies like antibiotics and evaluation concerning their biocompatibility and effectiveness in rats. **Acknowledgements:** The study is funded by Novartis Pharma AG.

P60 (EI0427)

Effects of Plasma Glow Discharge on Chemistry of Pmma

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Objectives: To examine the effect of plasma parameters on the surface chemistry of PMMA. **Methods:** Polymethylmetacrylate (PMMA) is very commonly use for dental applications. In this study PMMA samples were modified by RF oxygen plasma with various powers (10 W-50 W-100 W) which were applied for different periods (5 min-15 min-30 min). The effect of these plasma parameters (power and time) on the newly created surface free radicals, surface chemistry, topography, contact angle and surface free energy were investigated by electron spin resonance spectroscopy (ESR), x-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM) and goniometer, respectively. **Results:** ESR analysis indicated the presence of peroxy radicals on the surface of the oxygen plasma treated PMMA. The intensities of the peroxy radicals increased with increasing plasma power and the application period. The chemistry was also altered. XPS analysis revealed the controlled introduction of functional groups such as carbonate and free carbonyl groups onto the surface of PMMA. Also the roughness of the surface was increased from ~2 nm to ~7.5 nm after 100 W-30 min oxygen plasma treatment. The surface free energy (SFE) was also altered and contact angle results showed that surface wettability can be controlled by changing the plasma parameters. The maximum total dose application (oxygen plasma with 100 W for 30 min) enhanced the hydrophilicity of PMMA by causing a decrease of water contact angle from 70° to 26°. **Conclusions:** Plasma glow discharge can be applied to polymric films to alter their chemical and physical properties.

P61 (EI0096)

3D Smart Composite Collagen/Ha/Pcl/Prp Scaffold Seeded with Mesenchymal Stem Cells for Bone Regeneration in Vivo

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Objectives: The aim of this study was to develop suitable composite scaffold for bone regeneration *in vivo*. **Methods:** We prepared Collagen type I (Col)/ Hydroxyapatite (HA) scaffold with Polycaprolactone (PCL) nanofibres to improve mechanical properties of scaffold. Scaffold was seeded with autologous Mesenchymal Stem Cells (MSCs) in osteogenic differentiation media (Group 1). We also prepared scaffold (Col/HA/PCL) seeded with MSCs in osteogenic differentiation media enriched with platelet rich plasma (PRP) as a source of growth factors (Group 2). Both groups of scaffolds were implanted to the rabbit femur condyles where critical size defect 6 mm in diameter and 10 mm ± 0.5 mm in depth was made. Empty defects were used as a control. 12 weeks later rabbits were sacrificed and the femoral condyles were examined by histological analysis. The samples were stained with hematoxylin–eosin (HE), van Gieson's staining, Alcian blue-PAS and Gömöri trichrome staining. **Results:** In Group 1 a histological analysis revealed induced production of fibrous tissue which gradually ossificated not only from margin of defects. However, better results were observed on scaffolds enriched with MSCs and with PRP (Group2). There was explicitly predominant direct production of bone trabecules in whole volume of defects. In empty defects massive blood coagulum were observed with new formed fibrous scar tissue. Ossifications begin from the margin of defect. From mechanical testing of scaffolds was obvious that the moduli of elasticity under compressive test significant increased at the Col/ HA/ PCL scaffold compared to Col/ HA scaffold without PCL nanofibres. **Conclusions:** PCL nanofibres increased mechanical properties of Col/HA scaffold and PRP improved bone regeneration. This smart composite scaffold enriched with PCL nanofibres, MSCs and PRP present new possibilities for bone defect regeneration. Aknowledgement: Grant Agency of Czech Republic (grant No. P304/10/1307), The Grant Agency of the Charles University (grant No. 330611,164010)

P62 (EI0237)

Time-Resolved Characterization of Platelet Deposition in A Stagnation Point Flow Chamber

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Objectives: Thrombus formation still poses a problem in the development of devices in the cardiovascular system. The process is determined by the components of Virchow's triad, which describes the interaction of blood, flow and surface. The objective of this work is the development of a method to quantify Virchow's triad. For this endeavor the stagnation point flow is chosen. **Methods:** For the experiments a stagnation point flow chamber was designed and manufactured. The blood flows through a bore perpendicular onto a flat plate. In a radial distance the blood is collected in an annular channel. This creates a stagnation point flow with axial symmetry but with radially varying shear rates. The flat plate is a microscopic cover slip made out of native glass. It permits a fluorescent video microscopy with an inverted microscope. The blood was drawn from voluntary healthy donors into standard syringes containing sodium citrate. To dye the platelets with calcein red-orange AM, platelet rich plasma is temporarily separated. With a flow rate of 18 ml/h the blood enters a microfluidic device to mix with adenosine diphosphate (ADP) (2 ml/h, 20 µM) to stimulate the platelets. The activated platelets deposit on the cover slip influenced by shear rate. **Results:** In the onset of the experiment the platelets deposit evenly, but shortly after this a preference of certain regions are observed. The region around the stagnation point attracts more platelets, while the stagnation point itself remains nearly platelet free. In addition a development of insular pattern of platelet deposition is observed. These insular depositions develop in flow direction with elliptic shapes in the beginning. **Conclusions:** With this method temporal resolved platelet formation can be observed using also alternative surfaces modifications. From the results thrombus formation can be modeled considering Virchows Triads.

P63 (EI0132)

Evaluation of Cytotoxicity in Vitro of Biodegradable Polylactide Fibers with Spin Finishes

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Objectives: Biodegradable fibers with controlled properties may meet the requirements for medical applications. Poly(lactic acid) (PLA) is a biodegradable linear aliphatic thermoplastic polyester. There were prepared PLA fibers with five type spin finishes which were assayed for in vitro cytotoxic activities. **Methods:** The PLA fibres were prepared by a two-step melt-spinning process. The PLA Polymer 6201D, fiber grade with nominal MFI = 15–30 g/10 min, a NatureWorks LLC product was used. PLA fibers were coated with 5 types of spin finishes: **PLA 24** with 2.4% of Glicerol Ph Eur, **PLA 25**–0,.40% of Lurol PL 801, **PLA 26**–0.61% of Stantex 6457, **PLA 27–**0.36% of Lurol PT-L216, **PLA28**- 0.62% of Estesol PF 790. The fibres with linear density 2.2–4.8 dtex, tenacity 35–39 cN/tex, elongation ~50 % were obtained.To determine if they can affect cells, line cultures L929 (ATCC CCL1) was used. The cells (2×10^6 cells/ml) were incubated with fibres for 24 h, 48 h and 72 h (37°C, 5% CO_2). Cell growth, morphology and viability were determined. **Results:** After 72 h incubation, the level of cytotoxicity of **PLA 24** fibers was 2 (% dead-38), **PLA 25**–3 (% dead-100), **PLA 27**–3 (% dead-100), **PLA 28- 0** (% dead-99), **control fenol**-3 (% dead-94), **L929-**0 (% dead-3). **Conclusions:** Fibroblast cultures after contact with the four of PLA fibres showed cytotoxity effects. The cells were dead with hanged morphologie and lower proliferation. The result of the testing of PLA fibers with Estesol spin finish did not show any cytotoxicity effects and may be promising candidate for medical applications. *Financial support by the project* "Biodegradable fibrous products", POIG.01.03.01-00-007-/08-00 *and* EU in the frame of IE OP financed from the ERDF*, is gratefully acknowledged*.

P64 (EI0213)

Cell Colonization of Polyurethane Surface in The Polish Extracorporeal Ventricular Assist Device in Long Term Usage

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Objectives: The aim of the investigation was an immunohistochemical analysis of biological material adhered to the inner surface of polyurethane blood chamber of the pulsatile extracorporeal ventricular assist device. **Methods:** The investigated material were pulsatile, extracorporeal VAD, obtained after heart support. The analysis was performed on samples obtained from internal part of VAD's blood chamber. Blood pumps with a working time from 3 to 104 days were examined. An immunohistochemical analysis of the blood contact surface was performed. To detect and differentiate biological material, EnVision method from DAKO and the following monoclonal antibodies against human antigens were used: (1) anti-fibrinogen, (2) anti-actin, anti-CD3(+), anti-CD34, anti-CD45, anti-CD61, and anti-CD68. **Results:** The investigated material had an organic character. It consisted of fibrin deposition and several cell types. The cell depositions were well organized forming small cell clusters of erythrocytes, granulocytes, platelets and the low differentiated cells with epithelial and fibroblast morphology. These low organized cells presented mainly monolayer appearance. The highest cellularity of the inner side of the polyurethane wall was observed in cases with long time of VAD working. **Conclusions:** Increased time of heart support with the extracorporeal VAD induces the organism into cellularization of artificial surfaces. Several cell populations on different levels of differentiation take part in the construction of a cell monolayer.

P65 (EI0069)

Experimental Investigation of Mechanical Compatibility of a Hernia Mesh

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Objectives: The success of the surgical operation depends in a great extent on the mechanical behavior of synthetic hernia meshes which are used in the abdominal surgery to repaire different types of hernia. The aim of this work is to evaluate the mechanical compatibility of hernia mesh and human umbilical fascia (UF) comparing their viscoelastic properties. **Methods:** Uniaxial stress relaxation tests on mesh specimens—Surgimesh (SM) and fascia samples with dimensions (10×70) mm were performed using testing device FU1000/E. Ten mesh samples were cut along the rows of loops and parallel to the column of loops. Seventeen samples taken from six human cadavers cut parallel to fiber direction and perpendicular to them were used in stress relaxation tests. The initial deformation was 4% and 5% at 1.26 mm/sec rate of elongation.The relaxation process were described by: elastic modulus E, equilibrium modulus E_{eq} when the relaxation process was completed and reduction of the stress $\Delta\sigma$. **Results:** Stress reduction for fascia samples vary between 37- 55 %, while for SM was 35–71%. The initial stress for UF was in range 0.11–0.76 MPa and for SM was 0. 34–0.59 MPa. The differences between values of the elastic and equilibrium moduli for both materials were not pronounced. The results reveal that the viscoelastic properties of Surgimesh were close to viscoelastic properties of umbilical fascia at chosen levels of strain. **Conclusions:** The stress relaxation curves revealed orthotropic and nonlinear viscoelastic behavior of investigated materials. Further relaxation tests at higher strain levels are required to determine completely the mechanical compatibility between this brand of hernia mesh and human umbilical fascia.

P66 (EI0026)

Biomimetic Modification of Surfaces by Thiolated Glycosaminoglycans

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To explore and exploit the bioactivity of glycosaminoglycans (GAGs) towards adhesive proteins, growth factors and cells covalent immobilization on biomaterials or sensor surfaces is required. Three different GAGs (heparin, hyaluronan, chondroitin sulfate) were covalently modified by a disulfide containing crosslinker as a precursor for thiol generation. The thiolated glycans should be used to coat bare gold or vinyl-terminated glass or silicon surfaces to guide the adhesion of cells. The thiolated GAGs were prepared by using a dihydrazide crosslinker that was attached to the carboxylate groups of the glycans backbone. To characterize the immobilization of the thiolated GAGs in terms of quantity and also surface morphology, ellipsometry and atomic force microscopy (AFM) were applied. The wetting properties of the surface after immobilization were studied by water contact angle measurements (WCA) to obtain further information about the degree of surface modification. Human fibroblasts (HF) were used to study the bioactivity of the modified surfaces. The successful immobilization of thiolated GAGs was confirmed via growing layer thickness observed by ellipsometry. AFM measurements also revealed the surface coverage with thiolated GAGs. Furthermore the reduced WCA of the modified substrate indicates the binding of hydrophilic polysaccharides. The cell experiments demonstrated a decrease of cell size and adhesion compared to glass and vinyl-terminated glass surfaces due to the hydrophilic nature of GAGs, which indicate a switch from nonspecific to specific adhesion mechanism. The results demonstrate that thiolated GAGs can be effectively immobilized on gold but also vinyl-terminated surfaces, which opens the way of one-step modification of biosensors with different GAG. These sensors can be applied to study the properties of the relevant glycans with their natural binding partners such as different type of proteins and cells. This work was supported by the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement no NMP4-SL-2009–229292 ("Find & Bind").

P67 (EI0381)

Electrospun Biodegradable Materials for Vascular Regenerative Medicine

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Objectives: There is a rising interest for the development of small-sized blood vessels substitutes. Several studies have been focused on the development of a biodegradable graft temporary able to substitute the blood vessels and allow their complete regeneration after a certain time. We tried to develop a biodegradable material, with optimal mechanical characteristics and the capacity to allow cells adhesion, differentiation and proliferation by electrospinning to obtain a nano-fibrillar scaffold starting from a polymeric solution. **Methods:** We report the in-vivo application on rats of two new electrospun biodegradable materials, specifically designed to create tubular structures. Both biomaterials can be functionalized with several polypeptidic and non polypeptidic active molecules (growth factors or drugs). In one case PHEA-PLA was co-spun with silk fibroin (Fibro-PHEA-PLA) by a parallel electrospinning process to obtain a scaffold with two different polymeric fibers. In the other case, PHEA-PLA was mixed with polycaprolactone (PCL-PHEA-PLA) to obtain a single spinning solution for the obtainment of hybrid fibers scaffold. The in-vitro assay showed colonization by fibroblasts in both material. The scaffolds were implanted in a dorsal fascial pouch on Winstar rats to evaluate their in-vivo biocompatibility and tissue integration. The scaffolds were removed at 7, 15 and 40 days after implantation. **Results:** The pathological findings showed that both material were totally absorbed after 40 days without any sign of inflammation. A neutrophilic reaction was predominant at 7 days, especially for PCL-PHEA-PLA alone, whereas a lymphocytic invasion was

showed at 15. At 15 days Fibro-PHEA-PLA showed a good cell adhesion with a low grade of inflammation. Cell adhesion was confirmed at SEM scan. **Conclusions:** This preliminary study showed a good biocompatibility property of the scaffolds that needs of further investigations. The capability of the materials to be functionalized, should allow us to guide the development of bioengineered vessels.

P68 (EI0145)

Biovam in The Rat Model: A New Approach of Vascularized 3D Tissue

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Objectives: A major obstacle in tissue engineering is to create a surgically implantable tissue with long-term viability. Several promising techniques have focused on biological vascularized matrices (BioVaM) with preserved vascular pedicles in the porcine model. However the handling of this model is time consuming and expensive. Therefore, our aim was to establish a biological vascularised matrix in the rat. **Methods:** Small bowel segments of Sprague Dawley rats (250 g) were isolated and perfused via cannulation of the superior mesenteric artery and the portal vein. All cellular matrix components were removed by sequential treatment with sodium dodecyl sulfate, sodium deoxycholate, and DNAse. Quality of decellularization was investigated by histology and potential residual DNA by spectrophotometry. Primary endothelial cells (REC) were isolated from the major vessels of Sprague Dawley rats. Cells were labelled with fluorescent cell tracker and injected into the vascular pedicles of the matrix. Attachment of endothelial cells was assessed using fluorescence microscopy of the whole mount. After one week of culture in a bioreactor, cryosections of the construct were analyzed immunohistochemically. **Results:** After decellularization of the matrix, macroscopic and histological examination demonstrated absence of cellular components with conserved matrix architecture. This was validated by immune-fluorescent DAPI, Laminin as well as HE-stains. Tissue content of DNA was reduced by more than 99%. REC were characterized by specific staining against eNOS and vWF. After injection into the matrices, RECs attached along the vessel walls including the capillaries of the intestinal wall. **Conclusions:** Rat small bowel segments harvested with intact vascular pedicles and associated vascular network can be successfully decellularized and reendothelialized *ex vivo*. This rat model is an inexpensive and easy to handle alternative and appears to be a promising approach for establishing vascularised tissue constructs.

P69 (EI0099)

Adhesion and Growth of Bone Marrow Mesenchymal Stem Cells On 3D Organic-Inorganic Composite Scaffolds

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Objectives: In this study, we report on the adhesion and growth of bone marrow mesenchymal stem cells on 3D scaffolds, which are fabricated using a novel composite organic-inorganic material by direct laser writing. We investigate the relationship between the scaffold chemistry and topology and cell adhesion and growth. Furthermore, we explore the potential of the fabricated 3D scaffolds in bone tissue engineering. **Methods:** The hybrid material comprised a silicon-zirconium inorganic network and pendant organic tertiary amine groups in different mole ratios. The material was prepared using methacryloxypropyl trimethoxysilane (MAPTMS), (2-dimethylamino)ethyl methacrylate and methacrylic acid as the polymerizable monomers, whereas zirconium n-propoxide Zr(OPr)4, and the trimethoxysilane groups of MAPTMS served as the inorganic network forming moieties. 3D scaffolds are fabricated layer-by-layer using direct laser writing of the organic-inorganic composite material, a technique based on multi-photon polymerization. For the in vitro study we use early passages (1–4) of bone marrow mesenchymal stem cells isolated from posterior iliac aspirates of donors. We investigate cell adhesion by confocal microscopy and scanning electron microscopy. For the quantification of cell proliferation we use the MTT assay. **Results:** Scanning electron microscopy images show a strong initial adhesion of bone marrow MSCs in the first 3 hours after seeding on the structured composite material. We visualize the actin cytoskeleton and vinculin adhesion points of cells grown on the composite material by confocal microscopy. Preliminary proliferation data indicate a significant increase in cell number after 7 and 14 d under conditions with 1% FBS. Our results indicate a novel organic-inorganic composite material, which can be structured into 3D scaffolds and display a high initial cell attachment and promote cell growth. **Conclusions:** The strong initial adhesion and proliferation of bone marrow MSCs on the 3D organic-inorganic composite shows a high potential of the material as scaffold for bone tissue repair.

P70 (EI0426)

Surface Modification of Polymeric Materials by Plasma Glow Discharge Application

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Objectives: The materials used for medical applications can be modified by glow charge plasma in the presence of an active or inert gas or by further processes by binding various molecules covalently to the surface after plasma activation. **Methods:** Oxygen plasma glow discharge was applied to different polymers such as polymethylmethacylates (PMMA), polyurethanes (PU), poly L-lactic acids (PLLA) and polylactide-glycolides (PLGA). For PMMA and PLLA, the effect of plasma parameters (power and application time) on the surface chemistry was examined by ESCA, AFM, and surface free energies (SFE) were calculated from the contact angles measured by goniometer. For PU samples, heparin with two different molecular weights was linked covalently after plasma activation and the effects on thrombus formation were detected by incubating the samples with human blood. **Results:** It was shown that surface free energy (SFE) affect the cell attchment and for PMMA and PLLA surfaces had the highest cell attachment when the SFE values were about 60 mJ/m^2. It was also shown that combination of antithrombogenic molecules like heparin to the surfaces of PU samples after plasma activation increased their antithrombogenic property. On the other hand, addition of micro or nano sized hydroxyapatite (HAp) particles on surface activated scaffolds made the surfaces more bioactive and osteoconductive, and enhanced the attachment of osteoblast cells to the modified surfaces. **Conclusions:** Plasma glow discharge is a technique used commonly to alter only the surface without affecting the bulk properties of materials. By adjusting the parameters, it is possible to change only the surface chemistry, or to cover the surface ith the required molecules.

P71 (EI0326)

Zro2/Pcl Hybrid Material Synthesized via Sol-Gel: Characterization and Release Kinetics of Anti-Inflammatory

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Objectives: Controlled-localized drug release systems offer several advantages over other delivery options: they may provide the desired constant drug concentrations at the delivery site, lower systemic drug levels, and a reduced potential for deleterious side effects. The aim of this study was to synthesize and characterize novel sol-gel organic-inorganic hybrid materials to be used for drug delivery applications. **Methods:** Organic-inorganic hybrid materials of class I based on poly(ϵ-caprolactone) (PCL 6, 12, 24 and 50 wt%) and zirconia were synthesized by a sol-gel method, from a solution containing zirconium propoxide, PCL, water and chloroform. This solution was mixed with a solution of H_2O/Ethanol/anti-inflammatory drugs (ketoprofen and indomethacin 5 wt% and 10 wt%). Release kinetics in a simulated body fluid (SBF) was subsequently investigated and the amount of drug released was detected by UV-VIS spectroscopy. The structure of ZrO_2 gel, PCL and ZrO_2/PCL hybrid materials was investigated by XRD, FTIR and solid-state NMR. **Results:** The structure of the hybrids is obtained by means of hydrogen bonds between Zr-OH group in the sol-gel intermediate species and carboxylic group in the repeating units of the polymer, as suggested by FTIR analysis, and strongly supported by solid-state NMR. The ZrO_2 gel and ZrO_2/PCL XRD diffractograms exhibit broad humps characteristic of amorphous materials, while sharp peaks, typical of a crystalline material, can be detected in the diffractogram of PCL. Drugs entrapped in the organic-inorganic hybrids were released with logarithmic time dependence, starting with an initial burst effect followed by a gradual decrease. **Conclusions:** The synthesis of amorphous organic-inorganic hybrid materials containing drugs, obtained by sol-gel methods, was performed to devise new strategies for controlled release dosage forms. The release kinetics demonstrates that the investigated materials supply high doses of the anti-inflammatory agents during the first hours and then a slower drug release. The increase in the percentage of drugs increases the speed release.

P72 (EI0391)

Hyaluronic Acid Microparticles as Injectable Drug Carrier for Knee Cartilage Repair: Effect on Articular Chondrocyte Behavior

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Objectives: Cartilage is a specialized connective tissue performing many essential functions in the musculoskeletal system. If left untreated, cartilage injuries can lead to early progression of degenerative osteoarthritis (OA). Ideally, the efficient treatment of OA will require not only therapeutics that will reduce the degenerative processes, but also that will promote regeneration of cartilage. The goal of this study was to produce hyaluronic acid (HA) microparticles as a carrier for the delivery of therapeutic or signaling molecules (e.g. soluble growth factors) that can regulate cell function and cartilage regeneration. We examined the effect of the microparticles on human articular chondrocytes (HAC) viability, proliferation, CD markers and gene expression. **Methods:** HA microparticles were produced by water-in-oil emulsion [1] and characterized by scanning electron microscopy. HAC were isolated from femoral condyles by several enzymatic digestions. Cells were cultured in direct and nondirect contact with microparticles, previously sterilized, for 1, 3, 7, 14, 21 and 28 days. MTT and DNA assays were performed to determine cell viability and proliferation. Real time PCR and Flow cytometric analysis (FACS) were performed over time to assess chondrocyte phenotype. **Results:** Microparticles with a regular circular shape and having diameters between 8 and 40 µm were obtained. In vitro culture with HACs showed that microparticles did not show any kind of cytotoxicity and consequent decrease on cell viability. FACS revealed nonsignificant changes on the expression of CD44, 90 and 105 over time. Expression of COMP, SOX9 and aggrecan (cartilage-specific genes) were upraised when in presence of HA microparticles after 7 and 28 days. Collagen type I, II were fairly constant in both conditions. **Conclusions:** Our results suggest that HA microparticles enhance the maintenance of the chondrogenic phenotype over time and can be used as an injectable drug carrier for cartilage regeneration. [1]—Weiliam et al, 2004

P73 (EI0168)

Antifeedant Effects of Gamma Radiation and Rosmarinus Officinalis to Larval Stage of Tribolium Castaneum (Col: Tenebrionidae)

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Objectives: In this study antifeedant effect of combination of gamma radiation and *Rosmarinus officinalis* L. essential oil as a bio-controlling safe method on larvae of flour weevil, *Tribolium castaneum* (Herbst) were studied. **Methods:** Doses of 100 Gy of gamma radiation and 0.5 and 1 µl/l disk of essential oil were employed and after 72 hours, nutritional indices were evaluated. Relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and the feeding deterrence index (FDI) as nutritional indices were evaluated. Treatments were assessed by flour wheat disc at 27 ± 1 ° C and of 65% humidity in dark condition. **Results:** Results showed that relative growth rate had significantly decreased ($P < 0.05$) by combination of gamma radiation and *R. officinalis* and severity of this reduction when doses increased was higher. Relative food consumption rate also decreased when gamma radiation and *R. officinalis* combined with each other and its value had convert relative with increasing of doses. Experiments showed that the use of gamma ray and *R. officinalis* alone had no significant effect on ECI and feeding deterrence effect of larvae and reduction was observed only when they combined. **Conclusions:** The results showed that using of gamma radiation and *R. officinalis* that induces antifeedant effect can be use as effective method in control of *T. castaneum*.

P74 (EI0158)

Oil Nano-Encapsulation by Coacervation Method on Nutritional Indices of *Tribolium Castaneum* (Col: Tenebrionidae)

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Efficiency of nano- encapsulated of *Artemisia sieberi* Besser on nutritional indices of *Tribolium castaneum* Herbst was tested in this study. Several experiments were designed to measure the indices such as relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and feeding deterrent index (FDI). Treatments were evaluated by the method of flour disk bioassay in the dark, at 27 ± 1 0 C and 65 ± 5% R.H. Several concentrations were prepared and 10 adult insects were introduced into each treatment. Then, ingested food and weight gained were measured three days later. Results showed that nano- encapsulated of *A. sieberi* oil was highly effective compared to *A. sieberi* oil, and significantly decreased the RGR and RCR. Moreover the nano-encapsulated of *A. sieberi* oil was more effective on FDI than *A. sieberi* oil.

Lung Support, Valves and Varia

P75 (EI0263)

Pulmonary Blood Flow and Pressure as Well as Arterial Blood Oxygenation Simulations in Ventilated Artificial Patient Supported by Continuous Rotary Blood Pump

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Objectives: Our previous study suggested insignificant impairment of pulmonary circulation during artificial ventilation if the inspiration time is smaller than the duration of 2–3 heart cycles. This was due to pulmonary blood volume periodic changes. The aim of this study is to analyze influence of left ventricular assistance by continuous rotary blood pump (RBP) on hemodynamic in ventilated, virtual patient. **Methods:** Virtual RBP was added to a previously elaborated hybrid (pneumo-numerical), cardio-respiratory system adapted to simulate left heart failure. Ventilation to perfusion ratio (V/Q) in different lung regions as well as courses of pulmonary resistance (R_p) alteration, pulmonary blood flow (Q_p) and pressure (P_p), alveolar partial pressure of oxygen and arterial blood oxygenation (SaO_2) were analyzed for various parameters of mandatory ventilation and different RBP speeds (various RPM values). **Results and Discussion:** Experimental courses illustrate that RBP flow influences V/Q: the greater the P_p value because of low RBP flow, the smaller the influence of hydrostatic pressure on regional R_p. In particular, if the RBP blood flow is low, R_p is smaller because of increased P_p, and thus if the hypoxic vasoconstriction is not present, Q_p through worse ventilated regions (the shunt) is greater causing a decrease in SaO_2. If, however, the hypoxic vasoconstriction exists, results may be different. **Conclusions:** Artificial patient makes it possible to analyze complex multifactor problems, which would be impossible (because of both physical and ethical limitations) in cases of real patient examination. **Acknowledgements:** The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement num. 248763 (SensorART Project).

P76 (EI0406)

A New Pulsatile Mock Loop for In Vitro Simulation of Heart Valve Procedures in Porcine Heart

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Objectives: *In vitro* tests can effectively support the development of new surgical techniques or cardiovascular devices for heart valve repair. A mock loop able to house an entire explanted porcine heart and subject it to pulsatile fluid-dynamic conditions was developed, in order to get real-time images of the valvular structures and to allow the performance of simulated surgical procedures. **Methods:** The left ventricle of an entire swine heart is connected to an external pulsatile pumping system, consisting in a piston pump which can replicate the systolic and diastolic flow waves. The afterload, connected to the ascending aorta, simulates the human systemic impedance. The preload is achieved through a reservoir, which passively fills the left atrium. Access for endoscopic imaging is in the apex of the left ventricle. The mock loop is equipped with transducers that measure the mitral and aortic flow rates and the pressures in the ventricle, aortic root and left atrium. **Results:** The experimental pressure and flow tracings well matched the typical physiological wave shapes. A mean flow of 3.50 ± 0.1 lpm was obtained. The average working pressure of the systemic impedance simulator was 105 ± 13 mm Hg, with a good correspondence with the typical physiological values. The pressure drops across the mitral (2.8 ± 0.2 mm Hg) and aortic valves (6.7 ± 1.9 mm Hg) are coherent with data reported in the literature. Video recordings showed the great potential of the system in the observation of the cardiac structures dynamics in pulsatile conditions. **Conclusions:** A mock loop for subjecting explanted porcine hearts to pulsatile fluid-dynamic conditions was designed and tested. The system allows surgeons to simulate heart valve repair procedures or to implant transcatheter valves in a familiar environment and directly analyse their effectiveness. Hemodynamic conditions were consistent with the physiological ones, and real-time videos showing left heart valves dynamics were acquired with endoscopic techniques.

P77 (EI0287)

Image-Based Automatic Method to Not Invasively Monitor the Heart Rhythm of Zebrafish Embryos

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Objectives: In the last years the zebrafish embryo has been suggested as an ideal model for cardiac research. The overall similarity between zebrafish and human in response to cardiotoxic drugs was demonstrated, for example, in drug-induced cardiac arrhythmia. For this reason, several methods have been developed to assess cardiac rate (CR) in zebrafish embryos. In this study, we present a simple, not invasive method that adopts (i) confocal microscopy (CM) image recordings of the embryo's heart combined with (ii) image processing, and (iii) spectral analysis to measure the CR of zebrafish embryos. **Methods:** Zebrafish embryos at 96 hpf developmental stage were mounted in 0.5% agarose and analyzed with a Leica confocal laser-scanning microscope. Not treated and treated-with-drugs embryos were investigated. Cine CM images visualizing the beating ventricle were captured. A software was developed to automatically estimate heart rate from dynamic images. The software consists of: (i) detection of the border of the ventricle, obtained applying morphological operations; (ii) calculation, over a selected 2D slice, of the ventricular area and of the blood cells within the chamber at each time frame. Time series were built from sequences of consecutive ventricular area and blood cells number variations during the cardac cycle; (iii) estimation of the CR from spectral analysis of both blood cells number and area variation time histories. **Results:** Zebrafish embryos CR was measured not invasively and automatically. Results were validated by experts who reviewed the acquired images by visual inspection. The spectral analysis of the time series extracted from image processing clearly show changes in the CR of the embryos under the effect of drugs. **Conclusions:** The not invasive, image-based, fully automated method presented herein allows to estimate zebrafish embryo animal model CR avoiding genetists and biologists to resort to visual inspection or invasive techniques.

P78 (EI0284)

Influence of The Mechanical Bi-Leaflet Prosthetic Valve Designs on The Flow Field and Accumulated Stress inside The Simulated Aorta

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Objectives: Design feature of the bi-leaflet heart valve and existence of valsalva generate complex flow field inside the aorta and may impose excessive stress on blood component and cause possible platelet activation. Experimental study was conducted to analyze the influence of the heart valve designs and installed orientations on the aortic flow field, turbulent stress distribution and accumulated stress on blood component. **Methods:** Three mechanical bi-leaflet prostheses, the St. Jude Medical (SJM), the On-X (OX) valves with straight leaflets and the MIRA valve with curved leaflets were tested inside the simulated aorta. Dynamic PIV system was employed to analyze the aortic flow. Accumulated stress level on a blood component was calculated by tracing of a particle's turbulent stress level history. **Results:** The SJM valve's peripheral orifices tend to deflect the flow sideway during accelerating flow phase, while newer designs, the OX and the MIRA valves deflect less. The SJM valve's central orifice flow shows slower velocity than newer valves, the OX and the MIRA valves, which show strong flow through all orifices. The OX valve generates simple jet-type flow while the MIRA valve generates strong turbulent flow field due to the curved leaflets. Tracing of stress level on a selected particle through the central orifice showed higher accumulated stress level for the MIRA valve than that of the OX valve. **Conclusions:** Newer valves show strong flows through all orifices. The MIRA valve with peripherally curved leaflets generates strong and turbulent flow field and resulted in higher accumulated stress than the OX valve.

P79 (EI0153)

Impairment of Alveolar Gas Exchange Caused by Lamp Oil Aspiration Quantified in A Physical Model

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Objectives: Alveolar-capillary gas exchange occurs swiftly through diffusion e.g. O_2 from the alveolar space to erythrocytes through various "nonnegligible" barriers: filmy surfactant layer, alveolar epithelium, interstitium, capillary wall, plasma, and erythrocyte's membrane. Serious problems are reported when children drink accidently lamp oil followed immediately by an aspiration or after vomiting resulting in a drastic deterioration of oxygen diffusion causing Severe lung complications such as dyspnea. In order to quantify the degree of this adversity, an alveolar model to study the effect of various typical lamp oils as a diffusion barrier depending on its physical properties such as viscosity, density, and surface tension. **Methods:** In an "alveolus-model-chamber," the alveolar gas diffusion is mimicked to examine different commercial available types of lamp oils properties e.g. low kinematic viscosities (<7 mm^2/s). Perfluorocarbons (FC43), which is a high-density inert fluid, is used as a blood substitute having the same range of oxygen solubility. A thin layer of the chosen oils is spread atop the FC43. The system is continuously flushed by air or pure oxygen. Water and surfactants were used as control fluids. A micro-oxygen sensor (UNISENSE) placed in the FC43 compartment to monitor continuously the PO_2 variations of different oils. **Results:** All types of lamp oils used demonstrate a significant decrease of oxygen diffusion. The amount of impairment depends on the physical properties of the examined oil. The lowest oil resistance amounted to 15 folds in comparison to the resistance of water. The presence of surfactant influenced the degree of resistance to the dynamics of Oxygen uptake. **Conclusions:** It is evidently shown that the introduced physical module is able to demonstrate the serious impairment of gas diffusion quantitatively for the investigated lamp oils. It is also important still to quantify the degree of impairment of CO_2 elimination in further model studies.

P80 (EI0233)

A Long-Term Monitoring System for Hypertension Patients—Results From Animal Trials

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Objectives: Hypertension is one of the most common cardiovascular risk factors, however, hypertension control still remains a great challenge. The purpose of our study was to evaluate a novel monitoring system for recording blood pressure, pulse rate and body temperature of hypertensive patients. The device is designed for minimally-invasive implantation into the femoral artery. **Methods:** The device was tested in a chronic setting in 12 sheep for a period of three to six months. The implantation of the sensor was accomplished by means of a dedicated sheath (PASIS) in the femoral artery under x-ray-control. A reference sensor positioned contra laterally was used to counter-check the measurement quality and validity of each sensor after implantation. Via regular readout measurements and CTs, proper functioning as well as the position of the sensor was controlled. Pathologic evaluation was made to investigate the possible ingrowth of the sensor tip and the sensor cable at the end of each trial. **Results:** Although the project is still in process, several interesting findings can already be reported. The pressure sensors in general deliver stable pressure history. However, the stiffness of the micro-cable as well as the implantation technique have an important impact on stable positioning of the sensor in the artery and thus on the validity of the pressure curves. Cables with low stability tend to slip out of the artery easily whereas high stiffness may injure the vessel. **Conclusions:** Achieved findings are promising and support the feasibilty of a stable long-term blood pressure implant to monitor hypertensive patients. However, there are still some challenges to overcome. Future work will analyze the influence of different cable designs and implantation techniques as well as the validation of ingrowth and explantability.

P81 (EI0217)

Preparation of a Completely Autologous Valved Conduit with The Open Form of Trileaflets (Type VI Biovalve)

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Objectives: In body tissue architecture technology represents a promising approach for the development of living heart valve replacements and preparation of a series of BIOVALVEs. To reduce the degree of regurgitation and increase the orifice ratio, we designed a novel mold for a type VI BIOVALVE. In body tissue architecture technology represents a promising approach for the development of living heart valve replacements and preparation of a series of biovalves. To reduce the degree of regurgitation and increase the orifice ratio, we designed a novel mold for a type VI biovalve. **Methods and Results:** The mold had an outer diameter of 14 mm for implantation in beagles, and it was prepared by assembling 2 silicone rods with a small aperture (1 mm) between them. One rod had 3 protrusions of the sinus of Valsalva, the other was almost cylindrical. When the molds were embedded in the subcutaneous of beagles for 1 month, the native connective tissues that subsequently developed covered the entire outer surface of the molds and migrated into the aperture between the rods. The mold from both sides of the harvested cylindrical implant was removed, and homogenous well-balanced trileaflets were found to be sepa-

rately formed in the open form with a small aperture at the 3 commissure parts inside the developed conduit, which had a thick homogenous wall. Exposure of the obtained BIOVALVEs to physiological aortic valve flow in beagles revealed proper opening motion with a wide orifice area. The closure dynamics were suboptimal, probably due to the reduction in the size of the sinus of Valsalva. **Conclusions:** We developed a marvelous BIOVALVE with near perfect valve function by greatly altering the design concept of the mold used to prepare the BIOVALVEs. The mechanical behavior of this BIOVALVE might allow its use as a living aortic valve replacement.

P82 (EI0127)

The Influence of The Bicuspid Aortic Valve on Aortic Root Disease. The Pulsed Doppler Ultrasound and Fluid-Structure Interaction Study

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Objectives: A bicuspid aortic valve (BAV) is the most common congenital heart defect that occurs in adults. The BAV has formed only two leaflets instead of the normal three leaflets of aortic valve. At the beginning, BAV is works correctly. After many years of life numerous complications such as aortic stenosis regurgitation, aortic insufficiency and infective endocarditis, aortic dissection, and aortic aneurysm are revealed, that require surgical treatment. The knowledge on the pathogenesis and consequences of defects, as a result of hemodynamic changes, is still incomplete. The current question is how much dysfunction of BAV is heritable and is what degree is caused by hemodynamic changes. Therefore we studied the influence of the biomechanical properties, the geometry, orientation and methods of correction of BAV on the hemodynamic parameters based on clinical data. **Methods:** The segmentation of clinical images (CT), both of the aortic arch with normal tricuspid aortic valve (TAV) and dilated aortic arch with BAV was used to create physical and computer 3D anatomical models. The hemodynamical and mechanical analysis of the complex interaction between the valve leaflets, aortic root, blood flow and blood pressures (flow pattern, turbulence, stagnation area, strain, shear stress, wall deformation) of aortic arch was carried out using both Pulsed Ultrasonic Doppler Velocimeter (PUDV) and fluid-structure interaction (FSI). **Results:** The BAV reveals a significant influence on the flow patterns and stresses behind the valves. The Reynolds Normal Stress (RNS) is almost six time higher than RNS for normal TAV valves. Aortic wall shear stress reaches a critical value, increasing the probability of aortic wall damage. The BAV also causes the development of asymmetry in the flow, turbulences and vortex behind common coronary leaflet. **Conclusions:** The abnormal blood flow patterns caused by BAV seems a very important factor in the development of aortic root disease.

P83 (EI0130)

A Pumpless Extracorporeal Lung Assist for Premature Neonates: The Aachen Neonatox

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Objectives: Gas exchange in premature neonates is regularly impaired by immaturitiy of the lung. But mechanical ventilation, which is vital to sustain oxygenation and CO_2-elimination, causes mechanical and inflammatory destruction of lung tissue. To date extracorporeal oxygenation is no treatment option, i.e., due to the size of available oxygenators and cannulas. We hypothesized that a substantial improvement in gas exchange can be achieved by maintenance of the fetal cardiopulmonary bypass and interposition of a suitable passively driven membrane oxygenator (in sense of an "artificial placenta"). **Methods:** From a range of catheters we chose 14 Ga. One-Lumen-Central-Venous-Catheters for cannulation. The ideal insertion depth was investigated on premature lambs (n = 6, 2452 g ± 1054, 134 days ± 2.7 gestational age (term: 150 days)) for maximum flow, resistance and viability. A requirement specification for the complete extracorporeal circuit was based on the collected data. **Results:** Based on first in-vivo results 70 mm catheter length was chosen for the following in vivo test series. An oxygenator with 0.09 m² gas exchange surface area and 12 ml priming volume (19 ml incl. tubing) was designed, produced and tested. In-vitro tests showed a typical gas exchange of 47 ml_{CO2}/l_{blood} and 53 ml_{O2}/l_{blood} at 80 ml/min blood flow and 160 ml oxygen flow. In-vivo a mean pCO_2 (pO_2) at the oxygenator inlet of 54 ± 21 mm Hg (49 ± 26 mm Hg) and at the oxygenator outlet of 34 ± 7 mm Hg (160 ± 64 mm Hg) at mean blood flow of 91 ± 35 ml/min resp. 33 ml/Kg/min was found. The animals were hemodynamically stable. **Conclusions:** In close cooperation between engineers and neonatologists we developed a small oxygenator and extracorporeal circuit which is suitable as a pumpless extracorporeal lung support for premature lambs. We regard this as one step towards the clinical application of the artificial placenta.

P84 (EI0093)

New Artificial Internal Organ to Control Atrial Fibrillation

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Objectives: In some previous papers, atrial fibrillation has been reported to difficult for control in a lot of cases with conventional therapy. For the development of the new therapeutic device for the atrial fibrillation, implantable cooling device was developed. An implantable cooling device had been consisted from Peltier element with cooling water supply and voltage current controller. Cooling surface would be attached to the surface of atrium. **Methods:** Fourth intercostals space had been opened after anesthesia with Halothane inhalation, and various sensors had been inserted. And AF was induced by the electrical current with battery. **Results:** As the results, AF was recovered to the normal sinus rhythm after cooling with developed devices in all six goats. The method of cooling with implantable device for the control of AF might be evident in these experiments. **Conclusions:** Smaller size cooling device has been under development aiming at totally implantable type. Catheter type cooling device for the insertion by the use of fiber-scope type is now under planning for the clinical application. This new type device may be able to become good news for the patients with uncontrollable AF.

P85 (EI0013)

Architecture Design of A Novel Separable Mold to Obtain Autologous Tissue Heart Valves "Biovalves" Non-Invasively

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Objective: We have developed the autologous tissue heart valved conduit "Biovalves" grown in the recipients' subcutaneous spaces, which were autonomically formed precisely according to the shape of the material molds by encapsulation with connective tissues. In this study, a novel separable mold was developed for noninvasive removing the molds, which were completely impregnated into the formed Biovalve tissues with complex 3-dimentional shape. **Methods and Results:** The mold was consisted of six main plastic parts. Two were tubular rods (14 or 16 mm in diameter), which bound three small hemisphere-shaped parts resembling the 3 protrusions of the sinus of Valsalva. The assembly was fixed with pole by insertion into the hole of the combined two tubes to prepare the molds. The molds were placed into the dorsal subcutaneous pouches of beagle dogs for 4 weeks or goats for 8 weeks. The harvested implants were completely encapsulated with connective membranous tissues. After cutting the both ends of the implants the impregnated molds were smoothly removed from each end by separating the molds into the parts without any damages to the tissues, resulting in the acquisition of the flawless Biovalves. The Biovalve conduit had 3 protrusions resembling the sinus of Valsalva. A membranous tissue with the shape of a closed trileaflet valve was formed as intended by its design. In vitro functional evaluation and in vivo implantation study is on going. **Conclusion:** A novel separable mold for noninvasive preparation of Biovalve was developed, which is one of major steps toward its clinical application.

P86 (EI0274)

Early Echocardiographic Predictors of Progression of Left Ventricular Dysfunction (LVD) In Hemodialysis (HD) Patients

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Objectives: In the HD course prevalence of LVD increases and its severity enhances. Our aim was to show changes in echocardiographic parameters which could be early signs of development or further deterioration of LVD in HD patients. **Methods:** Echocardiography (two-dimensional, pulsed-wave, continuous wave, tissue Doppler) was performed in 48 patients (27 men, age 63.6+/–15.1 years, HD vintage 40; 5–154 months) before and after HD session at beginning and at the end of the 6 month study using Pro-Sound 4000 (Aloka, Japan). Kt/V and laboratory parameters were simultaneously evaluated. Unfavorable differences in echocardiographic parameters, occurring in patients without a change in classification of left ventricular function (LVF) over a study period were assumed to be early predictors of development or deterioration of LVD. **Results:** During 6 HD months in 31 patients LVF remained stable, in 12 deteriorated, and in 5 improved. Even in patients with stable LVF, left atrial (LA) diameter (42.0; 36.5–44.0 vs 44.0; 39.0–46.0 mm, p = 0.003) and LA area (19.3; 16.3–21.9 vs 20.0; 17.0–23.5 cm^2, p = 0.013) were greater before HD

session, and the HD session induced differences in LA diameter (1.0; 0.0–2.5 vs 3.0; 1.0–4.0 mm, p = 0.014), LA area (1.30; 0.35–3.22 vs 3.02; 0.69–0.74 cm^2, p = 0.028) and right atrial (RA) area (1.01; 0.02–2.38 vs 2.03; 0.70–3.43 cm^2, p = 0.040) were greater at the end of the study. Ultrafiltration (2155+/–926 vs 2177+/–952 ml, p = 0.666) and inferior vena cava diameter (22.8+/–2.6 vs 22.7+/–2.9 mm, p = 0.764) were not different. Differences in LA and RA diameters (among others) were also shown in 12 patients with worsening in classification of LVF. **Conclusions:** Increasing atrial diameters under stable hypervolemic conditions before HD session may be the early predictive signs of deterioration of LVF in HD patients. Adequate HD sessions with proper ultrafiltration can compensate these early echocardiographic changes, indicating worse tolerance of increased preload.

P87 (EI0126)

Comparison of The Added Compliance and Added Resistance Methods for Lung Function Tests

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Objectives: Noninvasive estimation of the total respiratory system compliance (Crs) and resistance (Rrs), which is not easy with present screening methods, could be useful in lung function tests. The purpose of this work was to compare added compliance (AC) and added resistance (AR) methods proposed to measure Crs and Rrs. **Methods:** Air is exhaled passively after the maximal inspiration and total respiratory muscle relaxation to AC (AC method) or briefly through AR to the atmosphere (AR method). Mouth pressure changes are analyzed to estimate Crs and Rrs. The AC method: Crs is calculated as AC*P1/(P0-P1) (P0, P1- the maximal and steady state pressures, respectively), whereas Rrs is estimated from the pressure decay time constant. The AR method: assuming that the mouth pressure is equal to the alveolar one when air does not flow, Rrs is calculated as AR*(P3-P2)/P2 (P2, P3—the pressures just before and after expiration cessation). As P2/AR is the airflow rate, the AR value does not have to be known, if a flow meter is used. **Results:** A suitable measuring system was developed. The methods were tested in the developed measuring system (one by one) and on several patients. Preliminary results showed that Rrs estimation is more trustworthy when the AR method is used (because of troubles with estimation of the time constant) whereas the AC method enables to estimate Crs in more reliable manner. The modification of the measuring system was done to apply the two methods together in turn. **Conclusions:** The proposed methods seem to be applicable under clinical conditions as a screening examination to support spirometry interpretation. Rrs estimation in both methods has shown different results. The measuring system and methods with the hybrid platform elaborated by our group can also be useful as a tool for research and educations.

P88 (EI0014)

110 Days of Extracorporeal Membrane Oxygenation in A Young Woman with Postpartal Cerebral Venous Thrombosis and Acute Respiratory Distress Syndrome

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Objectives: Extracorporeal membrane oxygenation (ECMO) is often the last resort for serious acute respiratory distress syndrome (ARDS) after all non invasive treatment options had failed to improve the patient's pulmonary condition. **Methods:** Here we present the successful long-term therapy with such an ECMO device over 110 days in a 28-year-old woman. She developed postpartal a cerebral venous thrombosis and severe respiratory insufficiency. **Results:** Veno-venous ECMO was able to rescue this young patient and can be the ultimate treatment option for patients with severe respiratory failure. **Conclusions:** Veno-venous ECMO was able to rescue this young patient and can be the ultimate treatment option for patients with severe respiratory failure.

P89 (EI0140)

Hemodialysis Arteriovenous Fistula Related Complications in Kidney Graft Recipients

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Objectives: The aim of this historical cohort study was to evaluate data on hemodialysis arteriovenous fistula (AVF) related complications in kidney graft recipients. **Methods:** The study cohort included 60 recipients of a kidney transplant with symptomatic AVF complications between January 2006 and April 2011. **Results:** From the 60 recipients (mean age 50 ± 10, range 14 to 73 years) 29 (49%) were males. Among all AVF, 45 (72%) AVF were located in the forearm (38 left), 8 (13%) in the upper arm (4 left), and 9 (15%) in the elbow (6 left). Complications occurred in 11.1% (60/538) of kidney graft recipients that were treated in our outpatient transplant unit during the study period. Average duration from renal transplantation to AVF complication occurrence was 46.5 months (range 1 to 209 months). The most common complication was painful thrombosis with or without thrombophlebitis, occurring in 28 patients (46.5%). Other complications were growing aneurysms (28%, 17/60), venous hypertension (7%, 4/60), distal hypoperfusion (7%, 4/60), high-output AVF with cardiac failure (5%, 3/60), trauma (1.5%, 1/60). Three patients (5%, 3/60) experienced problems in the AVF area not related with AVF. A total of 37 surgical interventions were performed in 35 patients (mean age 50 ± 12, range 28 to 73 years). AVF closures were performed in 16/35 (46%). Furthermore, extirpations of aneurysms were performed in 10/35 (28%), extirpation of thrombosed AVF in 1/35 (3%), simple thrombectomies were performed in 5/35 (14%) and thrombectomies with reanastomosis in 2/35 (6%). The majority of surgical interventions were performed by interventional nephrologist, under local anesthesia. **Conclusions:** Significant number of kidney graft recipients have complications related to AVF, often requiring surgical intervention. The most common complications are thrombosis, often with thrombophlebitis, and growing aneurysms. Vascular access related complications after kidney transplantation should be the focus of further studies.

P90 (EI0398)

Segmentation and Grid Generation for Numerical Simulations of VAD Connections with Patient-Specific Data

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Objectives: We are interested in the numerical simulation of the anastomotic region comprised between outflow canula of LVAD and the aorta. Segmentation, geometry reconstruction and grid generation from patient-specific data remains an issue because of the variable quality of DICOM images, in particular CT-scan (e.g. metallic noise of the device, non aortic contrast phase). We propose a general framework to overcome this problem and create suitable grids for numerical simulations. **Methods:** Preliminary treatment of images is performed by reducing the level window and enhancing the contrast of the greyscale image using contrast-limited adaptive histogram equalization. A gradient anisotropic diffusion filter is applied to reduce the noise. Then, watershed segmentation algorithms and mathematical morphology filters allow to reconstruct the patient geometry. This is done using the InsightToolKit library (www.itk.org). Finally the Vascular Modeling ToolKit (www.vmtk.org) and gmsh (www.geuz.org/gmsh) are used to create the meshes for the fluid (blood) and structure (arterial wall, outflow canula) and to a priori identify the boundary layers. The method is tested on five different patients with left ventricular assistance and who underwent a CT-scan exam. **Results:** This method produced good results in four patients. The anastomosis area is recovered and the generated grids are suitable for numerical simulations. In one patient the method failed to produce a good segmentation because of the small dimension of the aortic arch with respect to the image resolution. **Conclusions:** The described framework allows the use of data that could not be otherwise segmented by standard automatic segmentation tools. In particular the computational grids that have been generated are suitable for simulations that take into account fluid-structure interactions. Finally the presented method features a good reproducibility and fast application.

P91 (EI0239)

An Auto-Regulation Unit for Left Ventricular Assist Devices

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Objectives: Left Ventricular Assist Devices usually run at a constant blood flow, disregarding physiological conditions of the patient. The objective of this work is to propose an autonomous regulation unit that varies the pump flow on the fly, depending on several parameters. **Methods:** A set of possible physiological signals were considered as input to the auto-regulation unit. In particular, two implantable pressure sensors, placed before the pump inlet and right after the pump outlet are used to measure the current blood pressure and flow. As soon as the pressure at the pump inlet varies, e.g. due to a physiological increase of the oxygenated blood request, the auto-regulation increases the pump speed in order to keep the flow constant. This architecture was preliminarily tested on bench with a Sinergy LVAD (Circulite GmBH) and two custom-made implantable pressure sensor catheters. The pressure sensors were provided by STMicroelectronics. The auto-regulation unit is based on a STM32 microcontroller and runs almost in real time. **Results and Discussion:** The auto-regulation unit was able to cope with a decrease in the inlet chamber pressure by adjusting the pump speed instantaneously. **Conclusions:** From the preliminary bench testing performed so far, it seems that the auto-regulation of LVAD speed allows a constant flow to be maintained. Next step will be to add other sensors as input to the auto-regulation unit and then try the whole system extensively, first on an animal model, and then on humans.

P92 (EI0166)

Modified open Circuit and Vacuum-Assisted Venous Return Reduces Blood Usage During Cardiopulmonary Bypass

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Objectives: To determine whether vacuum-assisted venous return has clinical advantages over conventional gravity drainage apart from allowing the use of smaller cannulas, shorter tubing and reduced priming. **Methods**: A total of 80 CABG operations were performed at our institution between July 1999 to December 2010 using vacuum-assisted venous return with small venous cannulas connected to short tubing. These were randomized with 80 CABG operations using conventional gravity drainage. Priming volume, hematocrit value, red blood cell usage, and total blood product usage were compared by means of multivariate analysis. **Results:** Priming volume was 780 +/– 140 ml for small-cannula vacuum-assisted venous return, 1300 +/– 88 ml for gravity drainage ($P < .0001$). Smaller priming resulted in higher hematocrit values both at the beginning of cardiopulmonary bypass (26% +/– 5% compared with 21% +/– 4%, respectively, $P < .0001$) and at the end (28% +/– 4% compared with 24% +/– 4%, respectively, $P < .0001$). Red cell transfusions were used in 12% of the patients having small-cannula vacuum-assisted venous return and 41% of the patients having gravity drainage ($P = .001$); total blood product usage was 15% and 61%, respectively ($P = .001$). Although postoperative blood loss, length of stay in intensive care unit was similar in both groups, the association of vacuum-assisted venous return with lowered blood product usage was confirmrd also inpostoperative period. **Conclusions:** Modified open circuit and vacuum-assisted venous return results in (1) higher hematocrit values during cardiopulmonary bypass and (2) decreased red cell and total blood product usage.

Vascular Access in Hemodialysis

P93 (EI0157)

Hypertonic Citrate Catheter Lock Causes Protein Precipitation

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Objectives: Between haemodialysis treatments catheters are locked with a locking solution. Because of its antimicrobial properties hypertonic trisodiumcitrate has become popular in Europe. This solution not only is spilled when injected, but is in part exchanged against whole blood due to its high density. Plasma proteins are therefore exposed to hypertonic triodiumcitrate **Methods:** During in-vitro tests with hypertonic citrate protein precipitation was observed. Subsequent in-vitro studies showed that this phenomenon occurs at concentrations exceeding 12 percent. When locks were aspirated in-vivo precipitated protein could be separated and analysed. The main constituent was albumin. During in-vitro tests protein precipitation could be observed visually in the lumen of a 2 mm tubing, which is equivalent to a catheter lumen. **Results:** Hypertonic citrate locks are exchanged against whole blood even up to the highest point in the catheter. During this process plasma proteins come into contact with hypertonic citrate and subsequently precipitate. They may also partially or totally occlude the catheter lumen. Literature search revealed data confirming precipitation of proteins by different salts, which were shown to have consistent effects on the solubility of proteins. 'Salting out of plasma proteins by sodiumcitrate' has become a common method for serum protein purification since the 19th century. Nevertheless, none of these papers has considered its relevance with respect to clinical application in haemodialysis patients. **Conclusions:** Hypertonic trisodiumcitrate lock solutions are potentially dangerous and may be the underlying cause for reported embolic complications in patients with central venous catheters

P94 (EI0177)

Delivered Blood Flow Predicts Quality of Life in Dialysis Patients

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Objectives: The diminished quality of life (Qol) plays causative role in adverse outcome in hemodialysis patients. The aim of this study was to elicitate the association between the delivered blood flow and the Qol. **Methods:** The SF-36 questionnaire was validated in 121 hemodialysis pts. Data was obtained from history, laboratory findings, and dialysis regime for the previous 3 years. The sociodemographic data: age, gender, socio-economic status, education level, marital status, family support and presence of sleep disturbance. After adjusting for initial failures, the rates of thrombosis of AV fistulas, as number of episodes per patient year, were calculated. Stenosis and corrections of fistulas were observed. Multivariate regression analysis was performed on Qol scores. **Results:** We found that older age, lower social status, sleep disturbance and poor family support were associated with significantly lower Qol scores. Diabetic patients and those living alone scored significantly worse on the Physical and Mental component score. The age ($\beta = -259, p = 0.003$), family support ($\beta = 0.215, p = 0.007$), sleep disturbance ($\beta = -226, p = 0.006$), and delivered blood flow ($\beta = 261, p = 0.003$) were the strongest independent predictive markers for the Physical component score. For the Mental component score the family support ($\beta = 189, p = 0.044$), sleep disturbance ($\beta = -226, p = 0.006$), and delivered blood flow ($\beta = 273, p = 0.003$) were the strongest predictors. Analysis of patients with lower blood flows showed significantly higher rates of fistula thromboses ($\beta = -233, p = 0.01$). Patients with fistula thromboses rates higher than 0.25 episodes per year scored significantly lower for PCS ($p = 0.042$). **Conclusions:** The sociodemographic factors are of major impact on the Qol. Lower dialysis blood flow deteriorates the Qol to a remarkable extent, potentially caused by higher rates of fistula thromboses. These data suggest that patients with lower blood flows should be closely evaluated because of higher risk of thromboses of fistulas and impact on overall Qol.

P95 (EI0025)

The Impact on Dialysis Adequacy When Dialyzing With Single Lumen or Dysfunctional Double Lumen Central Venous Catheters

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Objectives: Double lumen (DL) central venous catheters (CVCs) often suffer from thrombosis and/or suction towards the vessel wall, both resulting in non-sufficient blood flow and a negative impact on solute removal during hemodialysis. Reversing the catheter connection often restores blood flows, but might lead to enhanced recirculation. The use of single lumen (SL) CVCs inherently leads to recirculation. We investigated the differences in dialysis adequacy using different settings of CVCs as vascular access, by evaluating total solute removal (TSR) of different solutes. **Methods:** A mathematical model was developed, combining a 2-compartmental model (simulating solute kinetics in the patient) and a dialyser model (simulating solute removal in the dialyser). The compartmental model was calibrated based on kinetic studies from literature for urea, phosphate (P) and beta-2-Microglobulin (β2M). Hemodialysis sessions of 4 hours were simulated in case of well-functioning DL CVCs (blood flow QB350 mL/min), malfunctioning DL CVCs (QB250), reversed DL CVCs (QB350 with 15% recirculation) and a 12 Fr SL CVC (effective QB273). TSR, calculated as solute mass in spent dialysate, was calculated for the different catheter settings and the 3 solutes. **Results:** For the well-functioning DL CVC, TSR is 787 mmol for urea, 43.8 mmol for P, and 310 mg for β2 M. If QB decreases, TSR decreases by 13% (urea), 14% (P), and 18% (β2 M), while reversing the catheter connection results in a TSR decrease of only 5%, 4%, and 1%. A 12 Fr SL CVC decreases TSR by 14%, 15%, and 18%. **Conclusions:** In case of malfunctioning DL CVCs, reversing the catheter connection restoring QB to 350 mL/min, does not impair TSR, even not with a recirculation of 15%. Using SL CVCs shows similar TSR results as malfunctioning DL CVCs with QB250 mL/min, such that SL CVCs might be an appropriate alternative in some clinical cases.

P96 (EI0373)

Impact of Hemodialytic Procedures and Dialytic Doses on Erythrocyte Glutathione S-Tranferase (E-GST) Activity

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Objectives: Glutathione-S-transferases (GST) represent a superfamily of ubiquitous enzymes devoted to the cell protection and they are thought to play a role in the detoxification of both endogenous and exogenous compounds. Previous study demonstrated an increased e-GST activity in uremic patients. Because of hemodialysis is a "detoxification" therapy, we hypothesize that there may be observe different e-GST activity levels with different techniques and/or dialytic doses in uremic population undergoing to dialysis. The aims of the study are to compare e-GST activity in normal and uremic subjects, and to correlate the dialytic dose and the hemodialysis technique (convective and diffusive) with e-GST activity. **Methods:** e-GST activity was assayed using a new automated procedure. 103 uremic patients divided into two groups basis on dialytic procedures 44 out of 103 patients underwent to standard bicarbonate HemoDialysis (HD-group); 59 patients were treated with online-HemoDiaFiltration therapy (HDF-group) 62 MHD patients and 80 healthy subjects (control group) were studied. **Results:** Comparing the e-GST activities of the control group (5.6 ± 1.7 U/grHb) versus all uremic patients (9.0 ± 3.1 U/grHb), we observed a significant statistically difference ($p < 0.0001$). Moreover, we observed statistical significant differences for e-GST activity ($p = 0.0036$), Kt/

Vurea (p = 0.0007) and weekly Kt/Vurea (p = 0.0004) in two subgroups of uremic patients. To try to distinguish between dialytic technique and Kt/V as the cause of the different e-GST expression, we divided all 103 hemodialytic patients in two subgroups using 1.3 as cut-off value of Kt/Vurea. In the patients with Kt/Vurea < 1,3 (n° pts) e-GST was 9,67 ± 3,23 while in patients with Kt/Vurea ≥1.3 was 8,65 ± 2,96, without any statistically significant difference (p = 0,156). **Conclusions:** This preliminary study will be confirmed by a large trial. In fact a very large number of patients need to highlight the eGST as a long term marker of detoxification, such as an "glycate haemoglobin" for the dialysis therapy.

P97 (EI0314)

Daily Salt Intake and Overhydration in Patients on Hemodialysis

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Objectives: With a progressive loss of diuresis, sodium and fluid restrictions are vital to control the extra cellular volume in patients on hemodialysis(HD). In anuric patients, each 8 g NaCl, requires 1 l of fluid intake to maintain normal serum sodium. If interdialysis weight gain (IDWG) exceedes 4–4.5% of dry weight (DW), overhydration (OH) can appear. **Aim:** to investigate the influence of daily salt intake (DSI) on signs of OH in patients on HD. **Methods:** In 75 patients on HD, mean age 58.5 ± 9,1 years, the DSI was calculated using the formula: NaCl(g/day) = 8*serum Na(mmol/L)/140(mmol/L) (mean weekly IDWG(Kg)*3/6.5). According to the median level of DSI, they were divided into low and high DSI patients. The total body water (TBW) was calculated using Watsons' formula. Therefore patients were followed for 6 months for: mean weekly IDWG,NaCl 20% given during HD, approaching the DW, hypertension (HT) and night dyspnea. Chest x-ray and heart ultrasound (HU) were performed. Evaluation for daily fluids intake(DFI), habits for salty food intake and habits for salting food with or without probe was made twice. **Results:** The median level of DSI was 12.14 ± 2.35 g/day. When age and TBW were dichotomized by mediana, higher DSI were found for age OR0.2:[CI 0.09–0.6],p = 0.009, patients with higher levels of TBW OR 6:[CI 2.16–16.75],p = 0.0001 and bigger DFI. The amounts of NaCl 20%, approaching the dry weight and collapses insignificantly affected the DSI p = 0.09;p = 0.169;p = 0.151, respectively). Powerful predictive factors for DSI like Na 20% (p = 0.0001, β = 0.245), total body water (p = 0.0001, β = 0.312) and DFI (p = 0.0001, β = 0.207) were found. **Conclusions:** Patients with high DSI have a higher risk of having more TBW. Older patients run a high risk of larger DSI. We must be careful with using 20%NaCl and patients must be educated for DFI. Since, low and high DSI patients don't differ in OH signs we propose investigation about difference in subcutaneous free of water storage of sodium in HD patients.

P98 (EI0196)

Contribution of Renal and Dialytic Clearances to Dialysis Adequacy Indices

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Objectives: The quantification of the respective contributions of continuous residual renal clearance (K_r) and intermittent dialyzer clearance (K_d) to total (renal plus dialytic) indices of dialysis adequacy is currently based on empirical rules. We explored whether a theoretical correct solution to this problem is possible for dialysis adequacy indices (DAI) by expressing them in a uniform way based on the assessment of the amount of solute (M_R) removed per one dialysis cycle (T_c = one week). **Methods:** Two types of DAI can be defined: (i) equivalent continuous clearance (ECC) and (ii) fractional solute removal (FSR) defined as M_R divided per: (i) reference solute concentration in blood and T_c, and (ii) reference solute mass in the body, respectively, with the "reference" meaning either: (i) peak, *p*, (ii) peak average, *pa*, (iii) time average, *ta*, or (iv) treatment time average, *trta*, values (Waniewski et al, 2010). Computer simulations were carried out for urea and creatinine applying a variable volume two compartment model of conventional, daily and nocturnal hemodialysis with constant solute generation rate, and K_r between 0 and 10 mL/min. **Results:** Using the definitions of FSR, ECC, K_d and K_r, one can derive the following formulas: $ECC_{ref} = g_{d,ref} \cdot (T/T_c) \cdot K_d + g_{r,ref} \cdot K_r$ and $FSR_{ref} = g_{d,ref} \cdot K_d \cdot T / V_{ref} + g_{r,ref} \cdot K_r \cdot T_c / V_{ref}$ (where T is treatment time, $g_{d,ref} = C_{trta}/C_{ref}$, $g_{r,ref} = C_{ta}/C_{ref}$, V_{ref} is reference volume, and ref = *p*, *pa*, *ta* or *trta*). The weighing coefficients $g_{d,ref}$ and $g_{r,ref}$ were estimated using computer simulations. The coefficients $g_{r,ref}$ and $g_{d,ref}$ cannot be both equal one for the same reference method. The calculated values of these coefficients were between 0.32 and 1.46. **Conclusions:** Renal and dialytic contributions to DAI can be added by applying weighing coefficients that depend on the dialysis schedule and reference method, but *not* on the type of DAI (ECC or FSR). These coefficients can be calculated using the reference concentrations during the dialysis cycle.

P99 (EI0120)

Survival of Hemodialysis Patients With Chronic Hepatitis C Virus Infection

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Objectives: The impact of hepatitis C virus (HCV) infection on mortality of hemodialysis patients remains controversial. The aim of study was to estimate the survival of HCV positive and HCV negative hemodialysis (HD) patients. **Methods:** We conducted prospective study with 149 HD patients followed from 01 January 2003 till 31 December 2010. Patient survival was estimated by the Kaplan-Meier method and compared by the log-rank test. The Cox proportional hazards model was used to estimate the risk of death in HD patients with HCV infection. **Results:** From 149 patients, 80 (53.7%) patients were men. Mean age of patients was 59.3 ± 13.8 years with mean dialysis duration of 34.1 ± 23.5 months. Hepatitis C virus infection was presented in 26.2 % (39/149) of the HD patients. HCV positive patients were characterized with significantly longer dialysis duration compared to HCV negative, (48.9 ± 21.6 *vs.* 28.9 ± 21.96 months, p = 0.000). During the study, 50 (33.6%) patients died. Cardiovascular disease was the main cause of death in 64% (32/50) of the dialysis patients. Patient survival was 56.4 % (22/39) for HCV positive and 70 % (77/110) for HCV negative patients. Log-rank statistics showed no significant difference in survival between HCV positive and negative patients. (log rank, p = 0.150). By Cox regression analysis, after adjusting for age, gender, and dialysis duration, HCV positive patients were characterized with a 0.43 fold higher chance to die compared to those without HCV infection (HR = 0.43, 95% CI 0.22- 0.83, p = 0.011) **Conclusions:** There was no significant difference between HCV positive and HCV negative patients in terms of survival, but HCV infection remains relative risk for mortality in hemodialysis patients.

P100 (EI0251)

Atherosclerosis and Its Risk Factors as Predictors of Femoral Neck (FN) Bone Mineral Density (BMD) In Hemodialysis (HD) Patients

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Objectives: There are some reports that atherosclerosis and its risk factors are associated with BMD. The aim of our study was to assess the possible association between atherosclerosis, its risk factors and FN BMD and bone metabolism seromarkers in HD patients. **Methods:** The study was performed in 60 HD patients (26 women, age 54.8 ± 15.3 years, dialysis vintage 36.9, 6.1–279.6 months). BMD was measured in the FN. Blood pressure, lipid profile and blood levels of homocysteine (Hcy), lipoprotein(a), phosphorus and bone metabolism seromarkers were evaluated. **Results:** HD patients with symptomatic atherosclerosis disclosed, after adjustment to age and waist circumference, lower T score (p = 0.010), BMD as % of young adults (p = 0.011), Z score (p = 0.027), BMD as % of age matched (p = 0.018). Next to age, presence of symptomatic atherosclerosis and total cholesterol (corr. $R^2 = 0.695$) or LDL-cholesterol (corr. $R^2 = 0.680$) were negative FN BMD predictors. Correlations between some bone metabolism seromarkers and atherosclerosis risk factors were positive **(**total alkaline phosphatase (ALP) with Hcy; tartrate-resistant acid phosphatase 5b (TRAP5b) with Hcy and phosphorus; C-terminal cross-linking telopeptide of type I collagen (CTx) with Hcy, phosphorus, diastolic and systolic blood pressures; osteocalcin (OC) with Hcy, phosphorus and diastolic blood pressure; osteoprotegerin (OPG) with phosphorus), of which ALP and OPG were negatively related to BMD. In the regression analysis, positive predictors for bone metabolism seromarkers, being negatively related to FN BMD, were found among atherosclerosis risk factors: for intact parathyroid hormone (corr. $R^2 = 0.731$)- phosphorus, triglycerides (TG); for ALP (corr. $R^2 = 0.889$)- Hcy, diastolic blood pressure; for TRAP5b (corr. $R^2 = 0.356$)- Hcy, phosphorus; for CTx (corr. $R^2 = 0.472$)- Hcy, phosphorus, TG; for OC (corr. $R^2 = 0.464$)- Hcy, phosphorus, diastolic blood pressure; for OPG ligand (corr. $R^2 = 0.894$)- Hcy. **Conclusions:** Symptomatic atherosclerosis and its risk factors by positive relation with some bone metabolism seromarkers adversely influence FN BMD.

P101 (EI0162)

Do Sodium Load and Fluid Intake Predict Quality of Life in Hemodialysis Patients?

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Objectives: The diminished quality of life (Qol) plays causative role in adverse outcome in hemodialysis patients. The aim of this study was to elicitate the most powerful predictors of the patients Qol among the sodium load and fluid intake. **Methods:** The SF-36 (Sort Form—36 Health Survey) that includes 8 different dimensions of health and two summary scores Physical Component Score (PCS) and Mental Component Score (MCS) were validated in 75 hemodialysis pts. Patients with decompensate heart failure were excluded. Sodium load was evaluated from daily salt intake (calculated using the formula NaCl (g/day) = 8*serum Na (mmol/L)/140(mmol/L)(mean weekly interdialysis weight gain (IDWG) (Kg)*3/6,5)), mean weekly amounts of NaCl 20% given

during HD, habit for salty food intake (1,5 g sodium in 100 mg food/ number of portions per day) and adding salt in food. **Results:** In the univariate analyses pts with habit for adding salt in food, collapses during HD and absence of edema had lover scores for mental Qol. Habit for adding salt in food, collapses during HD and absence of edema remained most powerful predictors of the mental Qol. In the univariate analyses for physical Qol, patients with habit for adding salt in food, salty food intake and absence of oedema had lover scores for mental Qol. All these factors remained most powerful predictors for physical Qol ($\beta = -0{,}230$, $p = 0{,}094$). Habit for adding salt in food, collapses during HD and absence of edema remained most powerful predictors of the mental Qol. **Conclusions:** The habit for adding salt in food, salty food intake and collapses during HD are associated with lover mental and physical Qol. Presence of oedema in patients with higher Qol we explain with better nutritional status. Other clinical signs for overhydration do not predicts the Qol in hemodialysis pts.

P102 (EI0282)

Efficiency of High Cut-Off Membrane Hemodiafiltration in Myoglobinuric Renal Failure

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Objectives: Treatment of myoglobinuric acute renal failure by convective dialysis techniques allows a higher removal of myoglobin (20 kD) than achieved by standard dialysis. High cut-off (HCO) dialyser membranes developed in last years allow the passage of molecules up to 60 kD molecular weight. We evaluated the removal of myoglobin by hemodiafiltration with the use of the HCO membrane. **Patients and methods**: Nine patients were treated by 14 hemodiafiltration procedures with high cut-off membrane (HCO HDF) for severe myoglobinuric acute renal failure. Rhabdomyolysis was caused by infection in two patients, and by a nontraumatic crush in three patients, it was statin-induced in two, and followed cardiovascular surgery in two patients. HCO HDF were performed with a high cut-off hemofilter at dialysate flow 500 ml/min, and blood flow within 250–300 ml/min, with citrate anticoagulation and postdilutional fluid substitution of 2–3 l/h. Albumin loses were replaced by infusion of human albumin solution. **Results:** Pre-procedure blood myoglobin concentration averaged 81 mg/l (range, 7.3–223.9 mg/l, mean, 58.2 mg/l). Serum myoglobin decreased exponentialy during the procedure with approximate half-time of 1 h. The myoglobin reduction ratio was 79.4% (range, 76% to 89%). An excellent clearance of 92 ml/min (range, 42–131 ml/min) was achieved. The highest cumulative amount observed of myoglobin removed into dialysate in the course of the procedures was almost 5 grams. Three of the patients died. Four of the survivors remained anuric for a period of 1–4 weeks, and mostly regained their renal function afterwards. The remaining patient begun to pass urine already at the end of the procedure. **Conclusions:** Highly efficient myoglobin removal by high cut-off membrane hemodiafiltration was demonstrated in our patients.

P103 (EI0101)

A Potential Use of Polyclonal Free Light Chain Levels for Monitoring in a Chronic Dialysis Population

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Objectives: Polyclonal free light chains (FLCs) are potentially an ideal marker for middle molecular weight uremic toxins. This study investigated whether FLC levels were influenced by treatment variables and residual renal function (RRF) in chronic haemodialysis populations. **Methods:** Polyclonal FLC concentrations were measured pre- and post-dialysis from two international centres. FLC concentrations were compared between different treatment modalities and RRF (>500 mls per 24 hours). Patients were excluded from analyses if they had experienced infection or significant illness within the previous 3 months. **Results:** Of the 112 patients recruited, sixty patients were anuric and 52 had RRF. The anuric patients had significantly increased total FLC levels compared with patients who maintained RRF (table). Patients between the two centres were comparable in terms of age, sex and vascular access, although patients at centre1 had higher CRP and FLC concentrations (table) than centre2 (CRP: (7.55 mg/L (0.12–170)) and 0.7 mg/L 90.3–6.6 respectively, $p < 0.001$ for both). Serum CRP concentrations did not correlate with the FLC levels, and no difference in CRP was observed between anuric and RRF patients. The use of HDF significantly increased the FLC percentage reduction from 39% (14–70) to 61% (43–81, $p < 0.001$). The vascular access (AVF or central venous catheter) did not affect the FLC concentrations (326.5 mg/L (70.95–738.5) and 280.7 (18.76–687.4) respectively). **Conclusions:** Polyclonal FLC concentrations are influenced by both residual renal function and dialysis modality. The measurement of FLCs could act as a simple marker for monitoring concentrations of difficult to remove middle molecule weight uremic toxins.
Table 1. Serum FLC concentrations (mg/L) [Median(range)]

P104 (EI0046)

Interleukin-18 (IL-18) Promoter Polymorphism In Relation To Gender of Patients Treated With Intermittent Hemodialysis (HD)

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Objectives: Serum concentration of IL-18 is increased in HD patients. The *IL-18* –1297C>T (rs360719) polymorphism may modulate the *IL-18* expression. The CC homozygote in IL-18 promoter increases IL-18 transcription. Our aim was to check differences in the *IL-18* –1297C>T polymorphism between HD patients and healthy controls, which could be at least a partial explanation for increased serum IL-18 in HD population. The potential influence of gender on the *IL-18* –1297C>T polymorphism was also analyzed. **Methods:** The frequency of *IL18* –1297C>T genotypes was identified by polymerase chain reaction-restriction fragment length polymorphism in 438 HD patients (190 women, 248 men) and compared to 421 (324 women, 97 men) controls. **Results:** The frequencies of –1297CC, –1297CT and –1297 TT genotypes were 4.8%, 44.0% and 47.9% in HD patients and 6.2%, 42.0% and 51.8% in controls. The p value for CC vs. CT + TT was 0.459, for CC + CT vs. TT was 0.905, and CC vs. TT was 0.530. The frequencies of –1297CC, –1297CT and –1297 TT genotypes were 5.3%, 42.1% and 52.6% in HD women and 5.2%, 40.4% and 54.3% in healthy women. The p value for CC vs. CT + TT was 0.994, for CC + CT vs. TT was 0.780, and CC vs. TT was 0.938. The frequencies of –1297CC, –1297CT and –1297 TT genotypes were 4.4%, 45.6% and 50.0% in HD men and 9.3%, 47.4% and 43.3% in healthy men. The p value for CC vs. CT + TT was 0.140, for CC + CT vs. TT was 0.317, and CC vs. TT was 0.110. **Conclusions:** The *IL-18* –1297C>T polymorphism is not gender-dependent. There is no difference in the *IL-18* –1297C>T polymorphism between HD and healthy subjects. Thus, differences in the *IL-18* –1297C>T polymorphism between HD and controls do not contribute to increased serum IL-18 in HD patients.

P105 (EI0052)

Health Related Quality of Life in Hemodialysis Patients with Different Type of Arterial Calcification Finding

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Objectives: There is a lack of studies investigated health-related quality of life (HRQoL) in hemodialysis (HD) patients depended of the type of the arterial calcification (AC) finding. The aim of this study was to evaluate whether presence of the different type of AC may impact HRQoL in HD patients. **Methods:** In a cross-sectional study we examined 88 HD patients (52 men; mean age 54.2 ± 11.8 years; HD duration 121.6 ± 72.4 months). Primarily, we evaluated the presence of arterial intima (AIC) and arterial media calcifications (AMC) using plain radiography of the pelvis. The scales for mental component summary (MCS) and physical component summary (PCS) were derived from eight different subscales originally developed for the short form health survey (SF-36). We compared PCS and MCS scores among the groups of patients with different type of AC (group without AC, group with AIC and group with AMC) presence on radiograms. **Results:** Patients (n = 33) with AMC finding on radiograms had lower ($p = 0.008$) PCS (42.58 ± 26.39 vs 55.83 ± 27.43), as well as lower ($p = 0.018$) MCS (44.76 ± 24.83 vs 52.66 ± 24.49) score in comparison with the group of patients (n = 25) with absence of AC. On the other hand, patients with the AIC (n = 30) finding on radiograms had higher ($p = 0.041$) only PCS (47.88 ± 26.22 vs 41.77 ± 28.54) score in comparison with group of patients without AC. We did not found differences in both PCS and MCS scores among the group of patient with presence of AIC and AMC. The groups did not differ significantly in variables that may affect the HRQoL of HD patients, such as age, gender, hemoglobin, serum albumin and dialysis doses. **Conclusions:** HD patients with AC finding on plain radiograms of the pelvis had lower HRQoL scores. This implies that clinical investigations aimed to preventing appearance of the AC in HD patients are still needed to improve patient quality of life.

P106 (EI0049)

K/Doqi Guideline Attainment of The Bone and Mineral Metabolism Markers and Relationship with Health Related Quality of Life in Hemodialysis Patients

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Objectives: The aim of this study was to evaluate whether K/DOQI guideline achievement of the serum mineral and bone disorder (MBD) markers may impact health-related quality of life (HRQoL) in hemodialysis (HD) patients. **Methods:** In a cross-sectional study we examined 82 patients (51 male; mean age 54.5 ± 23.9 years) dialyzed on average for 118.7 ± 61.3 months. The scales for mental component summary (MCS) and physical component summary

(PCS) were derived from eight different subscales originally developed for the short form health survey (SF-36) questionnaire. The proportion of the MBD K/DOQI guidelines achieved markers taken from the last 12 months measurements were compared among the groups of patients with various PCS (cut off = 43) and MCS (cut off = 51) levels. **Results:** Patients (n = 42) with PCS > 43 had significantly higher percentages of attained K/DOQI recommended levels for corrected serum calcium (Ca) (311/414, 75.1% vs 156/397, 39.3%), serum phosphate (P) (352/415, 84.8% vs 191/402, 47.5%) and serum intact parathyroid hormone (iPTH) (33/71, 46.5% vs 12/63, 19.1%) in comparison with the patients with PCS < 43. On the other hand, patients (n = 44) with MCS > 51 had significantly higher percentages of data attainment for serum P (364/439, 82.9% vs 179/378, 47.4%) and iPTH (32/75, 42.7% vs 13/59, 22.0%) in comparison with group of patients with MCS < 51. There was no difference in the attainment of the recommended levels for corrected serum Ca among the group of patients divided according to the MCS. The groups did not differ significantly in Ca × P levels, as well as in variables that may affect the HRQoL of HD patients, such as age, gender, hemoglobin, serum albumin and dialysis doses. **Conclusions:** Our evidence shows that greater HRQoL could be accomplished if a higher proportion of the K/DOQI recommended serum MBD levels is achieved.

P107 (EI0040)

How to Measure the Dose of Dialysis? Formal Urea Kinetics Versus Approximation Formulae

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Objectives: Kt /V is generally accepted as a parameter which describes the individual dose of dialysis as the product of urea clearance (K) and duration of a treatment (t) related to the urea distribution volume (V) of a patient. Dialysis dose Kt/V can be determined either using a formal urea kinetic model or approximation formulae derived from formal urea kinetic model. **Methods:** Data were processed from pre-, post-urea concentrations in a group of 102 patients dialyzed in Dialysis Centers in Aachen and Remscheid /Germany. Kt/V was calculated using the formal urea kinetic model of Stiller&Mann and the second generation Daugirdas (II) approximation formula. The Stiller/Mann model is calculated as a two-compartment model in two versions. The first one considers V estimated by the Watson formula (*St&M I*). The second one includes residual renal function, frequency of dialysis and urea distribution volume measured by bioimpedance (*St&M II*) (BodyScout Fresenius Kabi GmbH, Bad Homburg, Germany). Residual renal function was measured as the daily amount of urine. **Results:** The differences between Kt/V values of *Daugirdas (II)* and *St&M II* are about 29–31% considering frequency of dialyses per week, 7.8% with residual renal function more than 1000 ml/day and, 2% and 3.17% in patients with body fat less than 13% and more than 35%, respectively. **Conclusions:** The approximation formulae are only applicable in three times dialysis therapy per week, residual diuresis less than 1000 ml and body fat between > 13% and < 35%. Since it is important for an individual patient to obtain an individual amount of artificial kidney therapy, in all cases where individual data deviate considerably from normal a formal urea kinetic model should be applied.

P108 (EI0023)

How Adequate is Mid Dilution Hdf in The Removal of Protein Bound Solutes?

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Objectives: Convective dialysis strategies either in pre- or post-dilution have been proven superior dialysis to remove protein bound solutes (PBS). The concept of mid dilution, combining pre and post-dilution, might be a promising alternative to the more classical concepts. Therefore, we compared the removal of PBS between post and mid dilution hemodiafiltration (HDF). **Methods:** The present crossover study included 14 stable hemodialysis (HD) patients. They were kept for 4 weeks on high flux HD with one session on either post or reversed mid dilution HDF during the midweek session of weeks 3 and 4, performed in random order. Blood and dialysate flows were 300 and 800 mL/min in both modalities, while dilution flow was 75 mL/min in post and 150 mL/min in mid-dilution. During the test sessions, partial collection of spent dialysate was done to calculate Total Solute Removal (TSR). Blood was sampled at the inlet and outlet blood line to calculate the extraction ratio (ER), and pre and post dialysis to calculate reduction ratio (RR) of PBS and the small water-soluble solutes urea, creatinine, and uric acid. **Results:** For the PBS, no differences were observed for the TSR, ER, and RR. Minor but significant higher ER was found with post dilution for urea (0.92 ± 0.02 vs 0.87 ± 0.04—P < 0.001), creatinine (0.92 ± 0.02 vs 0.88 ± 0.02—P < 0.001), and uric acid (0.84 ± 0.02 vs 0.82 ± 0.03—P = 0.009). The combination, however, of this higher ER with a faster decrease in blood inlet concentration with post dilution HDF, resulted in a TSR and RR which were not different from those with mid dilution HDF. **Conclusions:** Since TSR was not found different in this cross-over study, post and mid dilution HDF were found just as adequate for the removal of small water-soluble solutes as well as of PBS.

P109 (EI0015)

Sodium Profiled HFR for Treatment of Drug Resistance Hypertension in RDT Patients

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Objectives: arterial hypertension is common complication of uremic patients; although the sodium and water balance should be corrected by dialysis treatment, many patients on regular dialysis treatment still need antihypertensive medications. the basic assumption of the present study was to asses whether an improved sodium balance could lead to better control of blood pressure. Methods: two male patients on RDT were severely hypertensive despite multiple therapy and appropriate dry weight as assessed by impedenziometria. they were treated for 9 months with HFR Aequilibrium (Bellco, Italy) (sodium and ultrafiltration profile) increasing the net amount of sodium removed per session without modifying the length of dialysis. **Results:** the first patient presented a slight reduction in blood pressure without changes of antihypertensive therapy; the second patient discontinued antihypertensive medications to normalize blood pressure after 8 months beginning of the study. **Conclusions:** the sensor for sodium is useful in evaluating the amount removed during dialysis treatment and may help improve treatment outcomes.

P110 (EI0007)

Nonadherence to Medical Investigations Impacts Mortality of Dialysis Patients

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Objectives: Compliance depends on the gender, race, occupation, education, income. Missed/shortened dialysis sessions, adherence to therapy, excessive phosphate levels and interdialyitic weight gains (IDWG) and smoking usually provide noncompliance indicators. **Aims:** to investigate nonadherence to medical investigations as noncompliance indicator and the impact on outcome of patients. **Methods:126** dialysis pts were scored for indicators of compliance from 0–2 and summary scores of were assessed. Patients with mean IDWGs >4.5% of dryBW and/or phosphorous level above 1.6 mmol/L were scored with 1, IDWG/BW more than 5.7% and/or 2.0 for phosphorous with 2. Patients were followed for 24 months up until death, transplantation or end of observational period. Compliance scores of survived and deceased patients were compared with Mann-Whitney test. **Results:** Estimated rates of noncompliance: medical investigations 63%, phosphorous 33%, IDWG/BW 22%, therapy 14%, treatment 9%. Noncompliance rate was 73%, adding adherence to medical investigations the rate rose up to 87%. We found the younger age (β = –0.294, p = 0.01), lower family support (β = –0.294, p = 0.01), lower education (β = –0.200, p = 0.025), smoking (β = 0.265, p = 0.003) and lower socioeconomic level (β = 0.365, p = 0.0001) with diminishing effect on the score. In the multivariate analysis, the younger age (β = –0.230, p = 0.008) was the most powerfull predictor of noncompliance. The compliance for medical investigations was predicted by lower income (β = –0.221, p = 0.018) and family support (β = –0.274, p = 0.008). After 24 months 23, (19%) pts died. Noncompliance scores were higher in the deceased population: medical investigations (p = 0.006), therapy (p = 0.022), dialysis regime (p = 0.029). The score for the dietary fluid, medications and treatment regimen was not found to be different between survived and deceased (p = 0.129), but when adherence for investigations was added the difference became significant (p = 0.047). **Conclusions:** The nonadherence to medical investigations has major impact on the mortality. More accessible ways to do crucial in-centre investigations, avoiding travel costs and need of companions which induce this non-compliance should be provided.

P111 (EI0342)

Vascular Access for Hemodialysis Patients in Slovenia—Data From The Slovenian Renal Replacement Therapy Registry

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Objectives: To present data on vascular access of hemodialysis (HD) patients in Slovenia. **Methods:** Data from the Slovenian Renal Replacement Therapy Registry, as of December 31, 2008, concerning vascular access are presented. **Results:** There were 1343 prevalent chronic HD patients (population of Slovenia is approximately 2 million). 58% were men, median age 66 years, range 9–94, mean 63 ± 15, 24.2% diabetics, median dry body weight 68 kg. Median weekly duration of HD was 13.5 hours, mean blood flow 283 ± 51 ml/min, 123 (9.2%) patients were dialyzed by single-needle dialysis mode. Vascular access were: native arteriovenous fistula (AV) in 82.4% (n = 1107), PTFE graft in 5.5% (n = 74) and HD catheter in 12.1% (n = 162). Position of fistula/graft was on forearm in 66%, elbow/arm in 33% and on thigh in 1% of patients. HD

catheters (n = 162) were: temporary (noncuffed) in 96.3% (n = 156) and permanent silastic in 3.7% (n = 6) of patients; precurved jugular in 78%, subclavian in 18% and femoral in 4%; single lumen in 80% and double-lumen in 20%. 30% citrate locking was used in the majority of catheters. The most common type of catheter used was precurved noncuffed 8 F single-lumen jugular catheter (Medcomp, Harleysville, PA, USA), either as single or two catheters in the same vein. There were 224 new HD patients in 2008, who were alive and on HD on December 31, 2008, median age 67.5 years, 29.5% diabetics. Their vascular access at the end of 2008 were AV fistula in 68%, graft in 3% and HD catheter in 29%. All catheters were noncuffed. **Conclusions:** 82.4% of prevalent and 68% of incident HD patients in Slovenia had native AV fistula at the end of 2008. The vast majortiy of HD catheters used were noncuffed single-lumen catheters with citrate locking, both for temporary as well as long-term use.

P112 (EI0341)

Vascular Access Activity in Dialysis Center Umc Ljubljana

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Objectives: To present vascular access activity in Dialysis center in University Medical center Ljubljana (UMCL). **Methods:** Data on hemodialysis catheters insertions, arteriovenous fistula (AVF) and graft constructions and revisions and Doppler ultrasonography examinations was evaluated from in-center medical archive. **Results:** Nephrologists in Dialysis center have constructed AV shunts from 1974–1982, AVF and grafts from 1974, inserted hemodialysis catheters from 1976, and performed Doppler ultrasonography related to AVF (preoperative mapping, Doppler of dysfuntional or thrombosed AVF and grafts) from 1992. Nephrologists also perform reconstruction and surgical salvage of the suddenly thrombosed AVF and graft. Pediatric AVF were also created. Surgeries are performed in the operative theatre at the Dialysis Center, mostly under local anesthesia, as outpatient procedure. In a few patients (mainly pediatric) AVF was created under general anesthesia. A total of 6,172 AVF/graft surgeries (creations/salvage procedures) and 21,740 of hemodialysis catheter insertion were performed by nephrologists in Dialysis Center UMCL from 1974 until the the end of April 2011. In 2009, 1,187 catheters were inserted, 267 AVF/grafts surgeries and more than 400 Doppler examinations were performed. The reason for the great number of catheters was in using 2 single lumen catheters instead of 1 double lumen catheter and providing the vascular access in the patients in intensive plasmapheresis (acute and chronic) and ICU (acute) therapy. **Conclusions:** From 1974 to April 2011 nephrologists performed 6,172 vascular access surgeries, mainly AV fistulas and grafts (re)constructions and inserted 21,740 HD catheters, with approximate yearly vascular access activity of 250 surgeries, 800 hemodialysis catheters insertions and 400 Doppler examinations.

P113 (EI0215)

Arteriovenous Fistula in Pediatric Patients with End-Stage Renal Disease: The Role of Preoperative Ultrasonography Mapping

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Objectives: To report our experience with ultrasonography preoperative mapping of arm and forearm vessels before arteriovenous (AV) fistula construction in pediatric patients. **Methods:** Between 2002–2011 preoperative ultrasonography examinations were performed in 24 pediatric patients with end-stage renal disease (ESRD), 15 boys and 9 girls, aged 9–21 years. Arteries and veins of both arms and forearms were examined by ultrasonography/Doppler preoperatively. Inner diameter of veins (under compression) and arteries and peak systolic velocity (PSV) were measured. Optimal position for AV anastomosis was suggested. If veins and/or arteries were not optimal, 8-weeks handgrip training was prescribed before the operation. AVF was constructed by trained nephrologist, under local or general anesthesia. **Results.** Suitable veins on right forearm were recorded in 21/24 patients, with internal diameter of 2.9 ± 0.7 mm. On the left forearm suitable veins were found in 22/24 patients, measuring 3.2 ± 0.6 mm. Internal diameter of right radial artery was 1.6 ± 0.3 mm, of the left 1.7 ± 0.3 mm, PSV 22 ± 6 cm/s on the right and 24 ± 5 cm/s on the left. In all patients AVF was constructed, in all but three construction was successful. Two patients with unsuccessful forearm AVF construction had suboptimal forearm vessels, one of them was on steroid therapy. In the third patient, there was a spasm of radial artery during operation, and after two primary failures in the arm, forearm AVF was successfuly constructed. On 1st of January 2011, 17/24 AVFs were still functional, double-needle dialysis was performed with average blood flow 256 ± 53 ml/min. Two patients were dialyzed using catheter (in one patient AVF did not mature and in one patient forearm AVF clotted). 5/24 patients had a functional kidney transplant (deceased donor). **Conclusions:** Ultrasonography can successfully assess arteries and veins of arms and forearms in pediatric ESRD patients, suggesting the optimal position for arteriovenous anastomosis, resulting in low primary failure rate.

Polymeric Membranes/Blood Interfaces

P115 (EI0362)

New Moulding Technique for Tissue Engineering of Fibrin Based Autologous Aortic Heart Valves

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Objectives: The presence of the sinuses of Valsalva in the aortic root is fundamental for the correct functioning of the aortic valve. Still the implementation of these features is not standard in tissue engineered valves. In our laboratory we aim at the realization of autologous heart valves starting from materials (fibrinogen and cells) isolated from the patient and shaped into 3D geometries like valved conduits with three leaflets by moulding techniques. However, when the diameter of the conduit is not constant as in the case of the aortic root including the sinuses of Valsalva, these moulding techniques cannot be straightforwardly applied and new concepts must be developed. We present a new fabrication method relying on a collapsible inner part of the mould that results in the realization of a heart valve scaffold recreating the complex geometry of the aortic valve. **Methods: The** new mould was designed with the 3D CAD software Pro/Engineer (PTC, Needham, MA, USA) and manufactured by rapid prototyping. The collapsible part of the mould was made of silicone rubber. The fibrin gel valves were produced by polymerizing a fibrinogen solution in TBS (10 mg/ml) with $CaCl_2$ and thrombin**. Results:** After the polymerization of the fibrin gel had occurred, the inner part of the mould was collapsed and the construct was successfully released without any tearing despite the poor mechanical properties of the hydrogel. We obtained a conduit presenting a three leaflet valve and the sinuses of Valsalva cast as a single piece without the need for suturing any of the parts together. Ongoing research is focused on the culturing and evaluation of the scaffold in vitro. **Conclusions:** Implementation of the sinuses of Valsalva is a crucial step towards the development of functional tissue engineered heart valves with optimal hemodynamic performance and reduced risk of thrombi formation.

P116 (EI0356)

Endothelization of Artificial Blood Vessels inside Large Bodies of Bacterial Cellulose Hydrogels

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Objectives: Macroscopic bodies of bacterial cellulose hydrogel with internal channels that mimic blood vessels were developed in our laboratories. The ability of human endothelial cells (HUVECs) to form an endothelial layer *in vitro* within these channels was examined. **Methods:** The characterization of macroscopic of bacterial cellulose hydrogels was performed by scanning electron microscopy (SEM). SEM was also used to evaluate the adhesion and proliferation of cells in the biomaterial; cell viability was evaluated by MTS and Live/Dead assays. The endothelization of internal channels of the hydrogel was observed by confocal microscopy by revealing HUVEC actin filaments (Alexa Fluor 546 phalloidin) and cell nuclei (DAPI). **Results and Discussion:** The formation of internal channels in the bacterial cellulose hydrogel was confirmed by SEM analysis. Channel walls are characterized by a high density of cellulosic fibers in the lumen side and a highly porous fibrous network in the surrounding hydrogel matrix. Seeded HUVECs adhered on the internal hydrogel channels and remained viable and proliferated for 26 days *in vitro*. By confocal microscopy we observed the formation of an endothelial layer on the lumen side. **Conclusions:** The internal channels formed within the macroscopic bacterial cellulose hydrogel can be an excellent platform for artificial blood vessel studies. The endothelization of these channels has proven its ability to maintain cell viability *in vitro* and can potentially be the basis of several biomedical devices development.

P117 (EI0338)

Liposome-Encapsulated Hemoglobin Ameliorates Skeletal Muscular Ischemia and Reperfusion Injury in The Rat

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Objectives: Liposome-encapsulated hemoglobin (LEH), a cellular artificial oxygen carrier, has been reported to ameliorate vascular ischemia and reperfusion injury in various organs. In the current study, LEH with low O_2 affinity (l-LEH, $P_{50}O_2$ = 45 mm Hg) was tested in acute disruption of limb perfusion as a simulation of thrombotic and/or atherosclerotic occlusion of the limbs, involving skeletal muscular ischemia and reperfusion. **Methods:** Physiological

parameters, such as blood pressure, heart rate, respiratory rate, tissue perfusion by Laser-Doppler flow meter as well as intramuscular PO_2, were serially monitored using dual PO_2 electrodes in the bilateral hind limbs in the SD rat. After baseline measurements, the left hind limb underwent ischemia by tightening a tourniquet for 70 min, followed 10 min later by intravenous administration of l-LEH (10 ml/kg, n = 4) or saline (n = 4) to the tail vein. Reperfusion was effected by relaxing the tourniquet and the rat was followed for an additional 50 min. Animals were sacrificed 7 days after ischemia/reperfusion for morphological analyses. **Results:** While PO_2 decreased precipitously after the onset of ischemia in the ischemic limb regardless of treatment, the PO_2 value in the contralateral intact limb showed a different pattern; decreased in the LEH-treated rats and increased in the saline-treated control animals. As the result, PO_2 ratio in the left ischemic limb to the right nonischemic limb (Lt/Rt ratio) tended to be higher during ischemia and became significantly higher in the LEH-treated rats than in the saline-treated control animals immediately after reperfusion to the end of observation. While plasma lactate, blood gases or electrolytes showed no difference after reperfusion, pathological studies 7 days later showed better muscular preservation in LEH-treated animals. **Conclusions:** The results suggest that early LEH-treatment may be protective of the skeletal muscle after ischemia and reperfusion in the rat.

P118 (EI0334)

An Atraumatic Mini-Pump for Pulsatile Flow in Bioreactors

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Objectives: Multiple types of bioreactors with perfusate are used for culturing and examination of cell cultures and their interaction with the perfusion media. In case of use with full blood or corpuscular blood components as perfusion medium, the traumatization of the necessary pump is a crucial aspect. The usual roller pumps creates unacceptable trauma and activation, due to the low priming volume and the multiple exposition of the blood particles to the pump shear. **Methods:** Multiple types of bioreactors with perfusate are used for culturing and examination of cell cultures and their interaction with the perfusion media. In case of use with full blood or corpuscular blood components as perfusion medium, the traumatization of the necessary pump is a crucial aspect. The usual roller pumps creates unacceptable trauma and activation, due to the low priming volume and the multiple exposition of the blood particles to the pump shear. **Results:** First tests showed a haemolysis increase for the MP of only 1.4 compared to 25,3 mg/dl/hour for the RP. ICAM-1, HLA-DR and CD11b were moderately activated by MP, but massively by RP. In contrast to RP, MP did not cause the expression of procoagulatory tissue factor on monocyte surface. **Conclusions:** A minipump with very cheap disposable components could be developed, which caused very low blood trauma in first tests.

P119 (EI0310)

Biofunctionalization of Blood-Contacting Materials for In Vivo Endothelialization

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Objectives: Biofunctionalization of blood-contacting materials with endothelial progenitor cell (EPC) specific capture molecules represents an auspicious strategy for in vivo tissue engineering of an endothelium on artificial implants. **Methods:** For this purpose, aptamers, antibodies, peptides, or magnetic molecules can be used as capture molecules for EPCs. **Results:** Synthetic graft surfaces coated with capture molecules for EPCs mimic a pro-homing substrate to fish autologous EPCs directly out of the bloodstream. After implantation, EPCs with high proliferation potential can cover the graft with a non-thrombogenic endothelium which maintains optimal haemostasis and minimizes the risk of restenosis. **Conclusions:** The realization of this in vivo tissue engineering concept and the generation of an endothelium on artificial surfaces could exceedingly enhance the performance of not only small caliber vascular grafts and stents, but also, in general all blood-contacting medical devices, such as heart valves, artificial lungs, hearts, kidneys, and ventricular assist devices.

P120 (EI0309)

Optimisation of Haemocompatible Stent Coating Using Poss-Pcu Nanocomposite Polymer

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Objectives: In-stent restenosis (ISR) is an unresolved issue in the field of interventional cardiology leading to vessel obstruction, reoccurrence of clinical symptoms and high mortality rates in a significant number of patients. Polymer coating of bare metal stents (BMS) has been applied with the intention of increasing stent patency rates and prolonging their maximum effective lifetime. In this study, we address key factors that directly influence the polymer coated stent performance, namely the selection of polymer, the applied coating technique and the adhesion of polymers to the stent surface. **Methods:** Cobalt Chromium BMS were coated with haemocompatible POSS-PCU using the ultrasonic atomization spraying technique. Surface coating procedures were optimised. Chemical modification and pre-polymer coating was applied for covalent modification of the BMS to improve polymer adhesion. The stress-strain behaviour, surface morphology, and wettability of 100 µm thick sprayed and casted POSS-PCU films were compared. Peeling tests and cyclic balloon expansion studies were evaluated. Pulsatile fatigue studies were performed over 400 million cycles, stimulating 10 year *in vivo* study, following ISO international standards. **Results:** Uniform surface coatings were achieved at 10 mm in thickness. Contact angle (θ) measurements show that POSS-PCU was more hydrophobic ($\theta = 96.7 \pm 2.47°$) than BMS ($\theta = 69 \pm 5.56°$). Evaluation of surface chemistry using ATR-FTIR showed no change in chemistry of the polymer after spraying. Peeling tests show a significant improvement in BMS-polymer adhesion after modification ($p < 0.0001$). Stress-strain behaviour revealed that maximum tensile stress was significantly lower for sprayed POSS-PCU ($p < 0.0001$) when compared with controls. SEM morphological assessments of the stent surface demonstrated that the spray coated stents maintained their physical integrity after fatigue studies. **Conclusions:** The optimisation of BMS coatings shows great potential in the development of new generation of high patency rate coronary artery stents and for coating of medical devices.

P121 (EI0290)

Morphology and Function of Smooth Muscle Cells on Poly (N-Butyl Acrylate) Networks With Different Elastic Modul

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Objectives: Small calibre vascular prostheses still lack medium and long-term patency. Inelasticity of the bulk material is one of the characteristics, which are implicated in the mechanisms of failure. Here we report about poly(*n*-butyl acrylate) networks (cP*n*BA) with adjustable elastic moduli, which can be tailored to match the E-modul range of human arteries (between 200 and 1100 kPa). In view of vascular protheses as potential application of cP*n*BAs, viability and functionality of primary human vascular smooth muscle cells (VSMC) on cPnBAs were explored. **Methods:** cP*n*BAs were synthesized by free radical polymerization. The elasticity was adjusted by varying the molar ratios of *n*BA monomer and polypropylene glycol dimethacrylate (PPGDMA) crosslinker. *In vitro* tests for cell viability were performed with VSMC (culture period of 96 hours). Deposition of VSMC extracellular matrix (ECM) proteins was quantified immunochemically. Secretion of cytokines was analyzed in a multiplex cytokine profile. **Results:** Two soft hydrophobic cP*n*BA networks with E-moduli of 200 and 1100 kPa (surface roughness in the wet state of 17 and 37 nm; $\theta_{advancing}$ of $123 \pm 4°$ and $111 \pm 4°$) were investigated. All *in vitro* tests suggested a decreased viability of the VSMC compared to the control, unaffected by the elastic modulus of the cP*n*BA. Furthermore, ECM deposition was decreased. The cytokine profile showed a shift towards the synthetic phenotype and, moreover, indicated a pro-inflammatory response of the VSMC to the cP*n*BAs. **Conclusions:** The results of this study demonstrate the challenge in the development of multifunctional materials. While the elastic modulus of cPnBA-networks could be successfully adjusted to that of human arteries, the tested polymers did not show an optimal performance as substrate material for VSMCs. Future studies aim at improving the biofunctionality by surface modification of these polymer networks.

P122 (EI0269)

Haemocompatibility of POSS-PCU Nanocomposite Polymers

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Objectives: There is increasing demand for synthetic polymeric materials that resist thrombosis for cardiovascular biomaterial applications. Recent advances in nanotechnology and polymer chemistry have led to enhanced physiochemical and mechanical properties of nanocomposite materials. In this study, we investigate the composition of nanomaterials by covalent attachment of polyhedral oligomeric silsesquioxane (POSS) nanocages in to poly(carbonate) urethane (PCU) to evaluate their effects on the coagulation cascade, platelet response and whole blood to evaluate haemocompatibility. **Methods:** POSS was reacted with PCU to form nanocomposite materials ranging from 2 to 8 % w/w. Polymer films were manufactured in to circular discs (16 mm in diameter). PTFE films were kindly supplied by Porex Technologies Ltd and used as a simple model control. Citrated whole blood was collected from healthy volunteers and centrifuged to isolate platelet rich plasma (PRP). Whole blood studies were conducted to evaluate thrombus formation through thromboelas-

tography (TEG) to monitor blood clot development, kinetics and lysis. **Results:** Morphological studies of adhered platelets on PTFE were composed mainly of spreading pseudopodia, dendritic structures and fully flattened activated phenotypes. On 2% POSS-PCU films only rounded platelets were apparent with no distinct pseudopodia. Whole blood studies revealed that clot formation was significantly reduced and less stable on 2% POSS-PCU films. TEG analysis also revealed a delay in clot formation, decrease in clot strength and increase in clot lysis when compared with controls. **Conclusions:** Covalent modification of POSS within PCU led to a distinctive anti-thrombogenic response. 2% POSS-PCU prevented platelet adhesion, aggregation and activation with a reduced rate of clot formation, stability, and increased lysis. This may be related to surface topography, chemistry, energy or charge on 2% POSS-PCU films. Such nanocomposite materials can be used for cardiovascular applications where thrombosis needs to be minimised and is currently undergoing preclinical evaluation following GLP protocols.

P123 (EI0199)

Analysis of Ceramic and Polymeric Membranes Using Fluorescent-Labeled Endotoxin

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Objectives: Endotoxins are components of the bacterial cell wall and are found in almost all fluids even in those which are poor of nutrients. This is a huge problem for medical and pharmaceutical applications, because already small amounts of endotoxin cause strong immune reactions. Endotoxin removal is possible by e.g. filtration. Therefore normally polymer membranes are used. The disadvantage of these membranes is their short lifetime. An alternative are ceramic membranes because they are inert and long-lasting. This work investigates polymeric and ceramic membranes concerning their ability of endotoxin removal from aqueous solutions e.g. dialysis water. Beside the analysis of endotoxin removal with conventional LAL-test fluorescence-labeled endotoxins and microscopic analysis of the membranes have been done. **Methods:** Several polymeric adsorber membranes and ceramic membranes were investigated concerning their endotoxin removal ability. Therefore membranes were loaded with aqueous endotoxin solutions (0–1000 EU/ml) and tested in cross-flow and dead-end modus. The permeat samples were analyzed by the limulus amoebocyte lysate (LAL) test. The endotoxin removal of a membrane was classified as sufficient at permeat endotoxin levels under 0.25 EU/ml. For microscopic analysis the membranes were loaded with fluorescence-labeled endotoxin, embedded and analyzed via fluorescence microscopy. **Results:** Although adsorber membranes showed good endotoxin binding capabilities the endotoxin removal was insufficient. Ceramic membranes showed significant better endotoxin separation. Microscopic analysis showed that in polymeric membranes endotoxin could penetrate until a depth of 25 µm, whereas in ceramic membranes the penetration depth was only 4 µm. **Conclusions:** Microscopic analysis of endotoxin filtration gives a deeper understanding in the separation behavior of the investigated membranes. Further fouling and capacity of the membranes can be observed directly. In the future a quantitative correlation of the endotoxin amount on the membranes should be established by measuring the fluorescence intensity of the labeled endotoxin.

P124 (EI0197)

Elastomeric Photopolymers by Thiol-Ene Polymerization for Vascular Tissue Engineering

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Objectives: Diseases of the cardiovascular system account for a significant number of morbidities and mortalities in developed counties. Hence, the need of suitable materials for artificial replacements for damaged blood vessels arose over the last decades; especially in the field of narrow blood vessel substitutes. This research focuses on the fabrication of blood vessel substitutes based on elastomeric photopolymers. **Methods:** Additive manufacturing technologies (AMTs) such as digital light processing are very capable techniques for the fabrication of constructs with complex geometries and high resolution mimicking the cellular structures of biological materials. For the application as blood vessel substitutes, polymers possessing urethane groups are interesting candidates since they exhibit elastic properties and also show good biocompatibility. Various urethane oligomer acrylates were tested in combination with different monofunctional acrylates as reactive diluents. In order to match the material properties of native blood vessels, a combination of low crosslink density and high urethane group concentration was desirable. To accomplish this, dithiols were added to formulations allowing the resulting thiol-ene reaction to compete with acrylate homopolymerization and thus lower crosslink density. **Results:** The high content of urethane groups caused a high density of reversible crosslinks due to H-bonds. With this polymer architecture the material had elastomeric properties comparable to native vascular tissue and exhibited similar tensile strength and suture tear resistance tests. Due to the hydrolytically cleavable ester bonds along the back bone and the branches, these polymers possess an inherent degradability similar to that of poly(lactic acid). The polymers also exhibited good endothelial cell attachment which is crucial for the long term performance of the vascular grafts. **Conclusions:** Structuring photopolymers by means of AMT enables the fabrication of artificial grafts with complex geometries. Implementation of the thiol-ene concept leads to materials with both highly elastomeric mechanical behavior and good biocompatibility.

P125 (EI0368)

2D Bi-Layer Scaffolds of Polycaprolactone and Chitosan β-Glycerol Based Film for Blood Vessel Constructs

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Objectives: The objective of this study was to develop a scaffold model aiming at fabricating small diameter blood vessel grafts with distinct surface properties. This study was designed to evaluate the influence of the scaffold properties on endothelial and smooth muscle cells. **Methods:** The scaffolds consisted of either a polycaprolactone (PCL) nanofibre mesh (NF) layer fabricated by means of electrospinning or a PCL membrane fabricated by solvent casting (SC); and a second layer prepared from a mixture of β-glycerol phosphate salt (GP) and chitosan (Ch). Scaffold characterisation was performed in terms of surface topography (SEM) and mechanical properties (tensile, Young's tensile and yield stress; and strain at break). For the biological evaluation endothelial and smooth muscle cells isolated from the vein of human umbilical cord (HUVECs and HUVSMCs) were used. Single cell cultures were established for both cell types and both scaffolds up to 7 days. Cell behaviour was evaluated after DNA quantification, alkaline phosphatase activity, methylene blue staining and SEM. **Results:** The tensile strength values for both SC PCL and NF PCL scaffolds exceeded the one of natural artery (15 MPa vs. 3 MPa vs. 1 MPa). As expected no alkaline phosphatase activity was detected in the cultures. Moreover, HUVECs attachment and proliferation rate was significantly higher on the SC PCL layer while for HUVSMCs the opposite was observed and the NF PCL layer was the preferable substrate for adherence and growth. **Conclusions:** Scaffolds with mechanical properties capable of withstand the physiological vascular conditions were obtained. The GP layer did not cause any sign of calcification which constitutes a good indicator for its incorporation within the blood vessel scaffold. The selective response of each cell type to a specific surface topography allows the definition of the design of a blood vessel graft combining HUVECs and HUVSMCs in the opposite layers.

P126 (EI0270)

Orientation of Electrospun Fibers by Minimizing Jet Instabilities

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Objectives: Basic electrospinning setups suffer from random fiber deposition conditions thus limiting their applications. The aim of this work was to minimize the bending instability of the jet in order to produce scaffolds with predesigned geometries. **Methods:** Polyurethane was electrospun on a horizontal oscillating and rotating conductive aluminum mandrel. The required electrostatic field essential to overcome the surface tension of the polymer solution was complemented by an auxiliary gradient field. This was generated by two additional electrodes that were symmetrically positioned around the spinning nozzle and operated with adjustable high voltage. The effect of the auxiliary electric field was characterized by comparing fiber deposition at different surface velocities, spinning times, with and without auxiliary electrodes. **Results:** Without the auxiliary electrodes only poor fiber alignment was possible. By introducing the auxiliary gradient field it was possible to minimize jet instabilities and improve fiber alignment. Fiber deposition took place in a focusable plane between the auxiliary electrodes. After 5 minutes spinning on a rotating mandrel the fiber deposition could be focused to a 3 mm width area. Oriented fiber deposition was achieved with the auxiliary electrodes at target velocities starting from 1 m/s for the first deposition layer. For longer lasting spinning durations surface velocities up to 6 m/s were necessary to achieve aligned and straight fibers. **Conclusions:** The bending instability, one shortcoming of electrospinning's controllability, could be considerably reduced.

Clinical VADs and Balloon Pumps

P127 (EI0378)

Predictive Role of Serum Cystatin C (Scys C) on Survival in Cardiac-Surgical Patients

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Objectives: Preoperative renal dysfunction is a known risk factor for morbidity and mortality in cardiac-surgical patients. Serum Creatinine (sCr) is the only renal marker in the preoperative scores, but it is known its diagnostic limitations on CKD. sCys C is a well recognized marker of early renal dysfunction, and previous studies suggest a sCys C predictive role for cardiovascular events in general population. However, few reports evaluate the prognostic role of sCys C in cardiac-surgical patients. Aim of this study is assess the long-term (two years) prognostic value of sCys C on mortality in adult cardiac-surgical patients. **Methods:** 421 consecutive patients (250 male and 171 female, mean age 67,72 ± 10,76 years) recovered in cardiac-surgery department from November 2005 to March 2007 were enrolled. We conducted a prospective observational study evaluating all causes of mortality until December 2009. At admission all patients were tested for renal function by sCr and sCys (normal value 0.5–0.92 mg/L). 217 out of 421 were submitted to coronary artery bypass graft (CABG), 150 valvular prosthesis, 54 for other kinds of cardiac surgery. Patients were subdivided in quartiles according to their sCys C values: Q1 sCys C < 0,81 mg/L (29 patients), Q2 sCys C 0,81–0,92 mg/L (81 patients), Q3 sCys C 0,93–1.10 mg/L (29 patients) and Q4 sCys C > 1.10 mg/L (282 patients). Kaplan–Meier cumulative survival curves were plotted for sCys C quartiles. **Results:** 124 patients (29,4%) reached the study end point. Patients in Q3 and Q4 showed a higher cumulative probability of mortality compared to patients in the lowest quartiles (p = 0,0007). **Conclusions:** Increased levels of serum Cystatin C may be considered a predictor of cardiovascular mortality at two-years follow-up in cardiac-surgical patients.

P128 (EI0286)

Minimally Invasive Implantation of a Para-Aortic Blood Pump

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Objectives: Mechanical support is the only way that is effective for breaking the vicious cycle of the heart failure mechanism. However, the decision of mechanical intervention via ventricular assist device (VAD) implantation has been deferred to end-stage heart failure because of the invasiveness and high mortality/morbidity rate of the surgery. Besides device efficacy, earlier intervention holds the key of reversing the trend of pathological myocardial remodeling. A counterpulsatile para-aortic blood pump (PABP) was invented to achieve this goal of early intervention. **Methods:** By taking advantage of the semirigid property of this PABP, a minimally invasive surgery (MIS) method was devised. This MIS procedure can be conducted using left thoracotomy without cardiopulmonary bypass support. A small incision size of 5–7 cm at the 5th and 6th intercostal space is required for the insertion of the PABP device. The implant site on the descending aorta was secured by a two-sided cross-clamping and the ligation of spinal arteries was done to free the aorta. A tool kit comprises a hole-maker, an insertion holder, a quick-release pouch, and an endoscope for visual monitoring was designed and constructed. With the aid of these tools and a specially designed surgical protocol, the present PABP can be implanted quickly requiring only 3–5 minutes ischemic cross-clamping time. A bandage type perivascular fastener was also invented and used to rule out the conventional need of anastomotic suturing. **Results:** It was shown on the animal model that this MIS PABP implantation can be accomplished safely and quickly and no bleeding complication was observed.

P129 (EI0283)

Preclinical In-Vivo and In-Vitro Investigations of a Thromboresistant Para-Aortic Counterpulsatile Device for Long-Term Ventricular Support

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Objectives: A para-aortic blood pump (PABP) intended for long-term counterpulsatile circulation support has been developed. This report summarizes the preclinical in-vivo and in-vitro tests that have been conducted for examining the hemodynamic performance and efficacy of this newly designed PABP. **Methods:** Acute porcine heart failure model induced by coronary ligation was adopted, and the hemodynamic support efficacy of PABP (n = 8) was evaluated using intra-aortic balloon pump (IABP, n = 8) as a benchmark. The chronic tests, designed to test the hemocompatibility and the integrity of the implanted device to major end organs of the recipients, were conducted using healthy calves (n = 5). No anticoagulants were administered during the post-operative 3-month period. To further look into the detail pulsatile blood pump flow field, a flow visualization experiment was launched in-vitro. Particle tracers were released to map and characterize the entire unsteady flow field. **Results:** When compared to IABP, PABP possesses better hemodynamic performance in every measured hemodynamic and metabolic index. For chronic tests, at autopsy, no clots were found on the pump surface and all recorded monitoring parameters including blood and organ functions are found normal, indicating the thromboresistance and biocompatibility of the PABP implant. The flow visualization using Lagrangian particle tracing reconfirms that no stasis zones exist and an excellent vortex washout effect was achieved in the blood pump chamber. **Conclusions:** Based on these in-vivo and in-vitro test results, it is concluded that the present PABP makes a promising counterpulsatile modality that is thromboresistant and therapeutic, and hence can be considered in the future as a viable long-term implantable ventricular assist device.

P130 (EI0159)

Pressure Measurements Along the Intra-Aortic Balloon (IAB): Implications of Operating at an Angle to the Horizontal

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Objectives: The aim is to investigate the operation of intra-aortic balloon (IAB) at angles to the horizontal, resembling patients being nursed at semi-recumbent positions. **Methods:** Two Datascope balloons, 34 cc and 40 cc, have been tested in a mock loop at 0,° 20° and 30°. Pressure at 7 positions along the balloon, and flow rate on either side of the balloon during inflation and deflation were sampled simultaneously at 2 kHz. The ratio (RQ) of water volume displaced towards the tip to the total volume displaced, pressure pulse generated by balloon inflation (PP) and the time of maximum pressure (TMP) at each location were determined. **Results:** At 0° TMP was reached at the tip and base of the 34 cc balloon almost simultaneously. At 20° and 30°, TMP was reached at the tip earlier than the base by 4 ms and 3 ms respectively. RQ at 0°, 20° and 30° are 51.5%, 48.8% and 49.6% respectively. TMP for the 40 cc balloon was reached at the tip earlier than the base by 4 ms, 4.5 ms and 6 ms at 0°, 20° and 30° respectively. This was associated with a decrease in RQ from 49.7% to 45.9% and 44.4% at 0°, 20° and 30°. Maximum PP along the balloon decreased by 12.57% and 18.6% at 20° and 30° for the 34 cc balloon and by 17.42% and 27.93% for the 40 cc. This was associated with a decrease in total volume displaced (TVD) of 2% and 21% at 20° and 30° (34 cc balloon) and 21% and 36% (40 cc balloon). **Conclusions:** TMP occurring earlier at the tip than other sections of the balloon provides a possible explanation on the reduction of RQ when balloons operated at angles to the horizontal. Efficacy of balloons maybe compromised when operated at angles to the horizontal with a reduction in both PP and TVD.

P131 (EI0238)

Stabilisation of Cardiac Function After Epicardial Biograft Implantation

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Objectives: Progress in cardiac tissue engineering is conditioned by the creation of a suitable environment for cells to build up an organised tissue. Hence, substrates need to be architecturally and chemically tuned to match the destination tissue. In this regard, micro-fibrous matrices, enriched with oxygen functional groups were designed and seeded with bone marrow derived mesenchymal stem cells (MSCs). In a rat in vivo model, the hypothesis that epicardial implantation of a cell-polymer graft has beneficial effect on cardiac function was tested. **Methods:** Microfibrous PCL nonwovens were produced by electrospinning and surface-coated by an RF plasma process (CO_2/C_2H_4 gas). MSC were characterised by FACS and 2 Mio cells were cultured for 7–10 days on the fibrous patches (10*15 mm). Cell mortality was assessed by LDH release, viability and morphology by MTT staining and SEM imaging, respectively. Two weeks post LAD ligation, Lewis rats with reduced ejection fraction (EF of 48 ± 8%) were randomised into 4 groups: MSC-seeded patches glued onto the infarcted area with Tisseel fibrin glue (n = 7), cell-free patches (n = 8), glue only (n = 4) and sham operation (n = 5). Echocardiography and pressure-volume loops were recorded after 28 days. Histological analyses are under investigation. **Results:** CD90+, CD45- and CD31- MSC spread on the matrix, producing a homogenous monolayer. The biografts allowed for safe implantation without signs of rejection, encapsulation or inflammation. Patches were permanently glued onto the myocardium, without adhesion to other

organs. Relative to pretreatment, MSC-seeded patches induced an EF stabilisation after 4 weeks (48 ± 10% and 48 ± 7% respectively). Cell-free patches resulted in a significantly decreased EF (45 ± 9% and 39 ± 4% respectively, p = 0.05). **Conclusions:** Preliminary data on EF analysis demonstrate that epicardial implantaion of plasma coated, MSC-seeded PCL biografts allow for safe implantation and attenuate cardiac remodeling. Further analysis will confirm eventual effect on myocardial regeneration and characterise macrophage invasion and vessel formation.

P132 (EI0219)

Large Animal Models of Heart Failure for Development of New LVAD Therapy: Combination Method of Micro-Embolization and Rapid Pacing Can Control the Degree of Heart Dysfunction and Stabilize the Result of Model Making

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Objectives: To develop the new LVAD therapy for heart failure, it is very much important to establish the method of making large animal model of heart failure. But unfortunately it is so difficult to make the model steadily with well controlled degree of heart failure. Now we have challenged to make large animal model with ischemic heart failure, more successfully by combination method of micro-embolization and rapid pacing. **Methods:** We studied an adult female goat (60 kg). From a multipurpose catheter introduced into the left carotid artery toward left anterior descending coronary artery (LAD), we injected the microsphere(size50 μm) into LAD 0.225 million(3,750/kg). 2 days later, we started rapid pacing (heart rate 200) and continued for 2 months. Aortic blood pressure (AoP), central venous pressure(CVP), arterial pressure-based cardiac output (= APCO, Edwards Life Science LLP, Irvine CA, USA) were monitored. We performed echocardiography as needed. **Results:** AoP was decreased from 120/80 mm Hg to 90/70 mm Hg, CVP was elevated from 6 mm Hg to 15 mm Hg, and APCO was decreased from 4.8 L/min to 2.8 L/min. She lost appetite, and was racked (roughly equivalent to NYHA III). But 2 days after, general condition was returned to NYHA II, and APCO was around 3. So we started rapid pacing, and cardiac function was remained to be low. By echocardiography, wall motion was hypokinetic in anterior wall and ventricular septum, ejection fraction(EF) was decreased from 80% to around 50%, and LVDd/Ds were dilated from 32/14 mm to 48/32 mm. Pathologically, there were many small areas of necrotic myocardium in subendocardium. **Conclusions:** By changing the rapid-pacing start timing and rate, according to the heart function after the micro-embolization, we can control the degree of heart dysfunction, which we can't achieve only with micro-embolization. And this may make the good survival rate of model making. This unique combination method of heart failure model-making may contribute for development of the new LVAD therapy.

P133 (EI0179)

Effect of Regional Left Ventricular Dysfunctions on Continuous Flow LVAD Assistance

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Objectives: Patients with severe heart failure are candidates for LVAD implantation. Left ventricular (LV) dysfunction is related to abnormal ventricular region (AVR) extension that could be affected by systolic abnormalities, diastolic abnormalities and electromechanical dissynchrony. The aim of this work is to study the effect of an AVR on hemodynamics during LVAD assistance. **Methods:** A lumped parameter model of the cardiovascular system was updated. Circulatory sections are described by Windkessel models. Atria, ventricular free walls and interventricular septum are described by variable elastance models driven by ECG times. LV free wall is represented by two variable elastance models to simulate an AVR. A model of parallel continuous flow LVAD was also implemented. Starting from a simulated pathological condition, the effect of LVAD on hemodynamics was studied changing: (i) AVR systolic elastance, (ii) AVR diastolic elastance (iii) the contraction delay between the two parts of the LV free wall and (iv) AVR extension. **Results:** All experiments compare the relative changes between pathological and assisted conditions giving their mean values. Variable that is more influenced by AVR changing is the external work. The presence of an AVR dissynchrony (0 ÷ 150 ms) influences mean aortic pressure (+10%). The changing of AVR systolic function (0.1 ÷ 1 mm Hg/ml) affects both LV end systolic volume (+6%) and cardiac output (+12%), while the changing of AVR diastolic function (0.01 ÷ 0.2 mm Hg/ml) affects left atrial pressure (–19%) and LV end diastolic volume (–14%). The presence of an AVR diastolic dysfunction could expedite the occurrence of right ventricular heart failure. This fact is more evident if a diastolic septum dysfunction occurs together with a moderate AVR diastolic dysfunction. **Conclusions:** The model could be useful to estimate the role of different parameters on LVAD performcan and could be used to support the clinical decision adapting the LVAD assistance to specific patient conditions.

P134 (EI0027)

Circardian Variation of Motor Current Could be Observed in Fixed Rotation Speed Centrifugal-Continuous Flow LVAD Support

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Objectives: The algorism for physiological control has been controversial in the patients supported with continuous flow LVAD, in which supplied blood flow is regulated by the pressure gradient between aortic and left ventricular pressure when operated at constant rotation speed. Little has known relating physiological control algorism, such as achieving physiological circardian rhythm. Motor current was evaluated in terms of the existence of circadian variation in dilated cardiomyopathy patients supported with centrifugal-continuous flow LVAD. **Methods:** The motor speed (rpm) and electric current (micro-ampere) data were collected every 10 minutes after device implantation and were divided in every 30 days data and calculated by least spectrum method. **Results:** Patients were 37.3 ± 14.8 years old, weighed 63.8 ± 15.1 kg at the time of operation. As of March 1, 2011, mean support duration was 1299.8 days (968–2124 days). Of the 6 patients, 3 received heart transplantation (at 1164, 1115 and 968 days of support) and 3 was still supported by LVAS with the mean support duration of 1517 ± 535 days of support. In all patients, the circardian variation of motor current was observed during almost entire course of LVAD support. The calculated periods of the circardian variation was 24.00 ± 1.00 hours. The amplitude of the circardian variation was 11.48 ± 4.50 μA. The circardian variation of the motor current was tended to be relatively low during night time whereas that was tended to be relatively high during day time. There were significant night and day time variation ($p < 0.01$). **Conclusions:** Circardian rhythm of motor current could be observed in fixed rotation speed centrifugal-continuous flow LVAD support. The cause and effect of this variation are still unclear although this is speculated to be correlated to physiological changes of some hemodynamic related parameters of patients.

P135 (EI0012)

Can We Generate Systemic Arterial Hypertension by Pulsatile LVAD in Our Patients?

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Objectives: On the basis of our personal experience of studying co-temporary scientific articles and the modeling of flow and pressure patterns in systemic vascular beds, we are encouraged to claim, that extremely small patients can experience systemic arterial hypertension generated by pulsatile LVAD. **Methods:** Our aim is to present our research about "iatrogenic systemic arterial hypertension produced by a left ventricular assist device." We want to show articles in medical journals supporting these facts and demonstrate our software application for the modeling of flow and pressure patterns for the confirmation of our thesis. We are able to prove the first author's hypothesis that different sized vascular beds are adjusted for the appropriate stroke volume of the native heart and so blood circulation in different sized vascular beds must be supported by the pump with an appropriate stroke volume. Thoratec VAD uses only one pump chamber size for all patients with different vascular bed sizes (65 ml). After implantation, all patients have the same stroke volume—65 ml, and the same average flow during the ejection period, i.e. 65 ml for 300 ms (13 l/min!). The average blood flow during the ejection period is higher in nonphysiological terms for extremely small patients and may cause iatrogenic systemic arterial hypertension. **Conclusions:** A safe and reliable ventricular assist device is a dream for many of us. Let's consider our work as a contribution for the better understanding of relationships between the human body and the mechanical device (VAD). We anticipate that the new generation of pulsatile VADs will come with adjustable stroke volumes and others parameters. The stroke volume expelled into the aorta must always be adjusted to the patient's size and actual requirements of the patient's body.

P136 (EI0194)

Physiological Control of an LVAD to Control Aortic Valve Motion in the Human Cardiovascular System

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Objectives: Continuous flow left ventricular assist devices (CF-LVADs) generally operate at a constant speed in the patient. In this mode, they change the systemic blood flow waveform significantly. If the left ventricular pressure is continuously less than the aortic pressure the aortic valve (AV) remains closed over the cardiac cycle. Blood flows through the CF-LVAD from the left ventricle (LV) into the aorta and pulsatility of the blood flow decreases. In this study, blood flow through the AV is controlled by applying a feedback control to the CF-LVAD to generate intermittent flow through the AV. **Methods:** PI control was applied to the flow rate through the LVAD in simulations. Minimum pump flow (Q_P) was set to 20 ml/s. Three flow ratios were simulated.

Q_p being equal to 1/3 of AV flow (Q_{ao}), Q_p equal to Q_{ao} and Q_p equal to $3Q_{ao}$. The ventricle was beating at 80 bpm with 45% / 55% systole / diastole ratio. **Results:** In the simulations the aortic valve duty cycle ($t_{open}/t_{cardiac\ cycle}$) did not change. However Q_{ao} changed significantly. The Q_p was controlled as desired by making the CF-LVAD operating speed variable over a cardiac cycle. The cardiac output value was 3.30 for all simulation protocols. **Conclusions:** Simulation results show that the AV duty cycle does not change for the same cardiac output values. However, change in the amount of the flow rate through the aortic valve indicates that AV valve motion changes. In other words, without compromising total systemic perfusion the AV motion can be controlled by applying variable pump speed control. However, Q_{ao} has to be estimated because it cannot be measured directly in a patient. Experimental validation will be carried out as a next step.

P137 (EI0065)

Outcomes for Continuous-Flow Left Ventricular Assist Device Patients Stratified by Severity of Clinical Status

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Objectives: The use of continuous-flow left ventricular assist devices (LVAD) is an accepted therapy for patients with advanced heart failure. New generation of these devices may have an impact on improved survival rates and quality of life (QoL). Here we report about our single-center experience with the new generation of LVADs. **Methods:** Between 2006 and 2010, 41 transplantable and 8 nontransplantable patients were selected for LVAD-therapy at our institution due to refractory severe heart failure. All patients were INTERMACS Level 1 to 3 (Level 1: n = 23; Level 2: n = 18 and Level 3: n = 8). The cohort included 44 men and 5 women with a mean age of 53 ± 12 (range 20–75 years). The patients were supported either by HeartMate II (n = 39) or HVAD (n = 10) LVAD. In-hospital (30-day) and long-term survival, freedom from reoperation and neurological complication, and rate of drive-line infection were examined. Additionally, the QoL was assessed. **Results:** Mean support time was 138 ± 53 days (range 1–867 days). In-hospital (30-day) mortality was 27% (n = 11) due to severe cardiogenic shock with multiple organ failure and sepsis. The follow-up survival for all was 32 of 49 patients (65%). Bleeding requiring reoperation occurred in 13 patients (n = 27%). Neurological problems were identified in 12 patients. There were 6 drive-line infections. 63% of all patients were discharged at home. Overall, 3 patients underwent transplantation, 23 patients awaiting a donor organ, 1 patient was successfully weaned and finally, 5 patients are on destination therapy with good QoL. **Conclusions:** Treatment of severe heart failure with new continuous-flow LVAD can significantly improve the acute and long-term survival with low device associated complication and improved QoL.

Tissue Engineering

P138 (EI0425)

Novel 3D Multilayered Coculture System for Investigating Stem Cell Differentiation

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Objectives: To develop an ideal coculture system for mesenchymal stem cells and articular chondrocytes. A number of recent studies show synergistic effects for chondrogenesis when the two cell types are cultured together, but there is still some ambiguity as to the specifics of these interactions. The critical paracrine signals and/or cell-cell adhesion modules involved must be identified. Via tailoring of the chemical and physical environment exposed to the cells in coculture, ideal conditions will be identified for promoting the synthesis of cartilagenous tissue. **Methods:** I intend to explore a novel technique for depositing layer-by-layer nanofilms of ECM molecules on individual cell surfaces to permit cell contacts analgous to native ECM interactions. QCM-D using adhered Extensive work on polyelectrolyte multilayers and hydrogel encapsulation of cells are routinely carried out in our lab and will be incorporated into a "3D" coculture system for the cells. Differentiation will be assessed with qRT-PCR, histology, and novel molecular reporters associated with up-regulation of chondro-specific genes. Cell types will be distinguished in situ via cell tracker molecules and/or other fluorescent labels. **Results:** An effective, 3D coculture system will be established for hMSCs and chondrocytes that will permit elucidation of the communication system between the two cell types. We expect to show that cartilage-specific genes are upregulated in constructs versus control groups. **Conclusions:** A recent trend in literature concerning cocultures involving adult stem cells is that, instead of promoting lineage specific differentiation, the stem cells rather provide support to improve the relevant function of the adult cells. This work will shed light on which soluble factors and/or cell-cell adhesions are involved in these processes and inform further tissue engineering strategies.

P139 (EI0418)

Investigation of Self Crosslinking Polyelectrolytes to Conformally Coat Individual Living Yeast Cells

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Objectives: One of the most promising applications of encapsulated living cells is their use as protected tissue for implantation in the human body. A suitable system for the immuno-protection of living cells is conformal coating. Layer-by-layer assembly, a commonly used method for conformal coating, uses sequential deposition of alternating layers of positively and negatively charged polymers to coat materials with functionalized nanofilms. This permits the preparation small capsules with minimal encapsulating material that helps to maximize metabolic exchange while minimizing overall capsule size. This work describes the use of auto crosslinking polyelectrolytes to coat individual living yeast cells. The effects of polymer properties such as molecular weight, concentration and compositions on cell coating and viability will be discussed and compared with non-crosslinked analogs. We also report preliminary results on encapsulated yeast cells internalized by the ciliated protozoan *Paramecium primaurelia*. This model system can serve as a tool to test for the protection capabilities against lysosomal enzymes. **Methods:** Bakers' yeast cells, *S. cerevisae* are conformally coated with auto crosslinking polyelectrolytes. The polymer shells are formed by successive electrostatic deposition of cationic polyamines and reactive polyanions capable of covalently crosslinking with the polycation. Fluorescent labels are used to map the distribution of both polyanions and polycations on the cell surface. **Results:** Successful coating of living yeast is reported, while maintaing cell viability. **Conclusion:** Encapsulation of living yeast cells allows us to create a model system whereby we can investigate the effects of polymer molecular weight and compositions on cell coating along with cell viability.

P140 (EI0419)

Stem Cell Bionics: Vision, Methodology and First Clinical Results

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The physiological mechanisms how stem cells are led into a three-dimensional regenerative process are presented as the basis of a bionic concept of stem cell therapy. This platform technology uses the wound as a triggering cofactor for in situ and in vivo technology as an alternative to in vitro stem cell theories and methods. The preparatory process is made possible by tissue specific bioreactors and highly standardized processes. In a diabetic wound the capacity of regeneration is significantly reduced or totally lost. We have developed a technology that topically activates this regeneration potential by endogenous stem cell activation and combines it with an ad hoc transplant of cells obtained from the peripheral blood of the patient. This approach intends to mimic the normal wound healing process while the homologous application of blood cells allows paracrine effects of the transplant that improve healing. The scientific rationale and technology focuses on an in situ and in vivo rather than a conventional in vitro use and induces an in situ boosting and commitment effect for stem cells that leads to an improved tissue regeneration. The elucidation of the underlying mechanisms, the positive regulatory environment and the safety of the process, the striking preclinical and pilot clinical results do warrant a further development in a multicentre clinical trials. Tissue regeneration in skin (burns, diabetic wounds) and bone defects has become a clinical reality. Apart from these areas other tissue applications including cartilage, liver, spinal cord injury, heart valves or trachea are being developed as well. Stem cells are the basis for regeneration and bioreactors are the instruments that make the respective biological technologies available for clinical therapy. Apart from mechanistic studies, preclinical animal trials and early clinical examples are presented.

P141 (EI0410)

In Vitro Performance of K-Carrageenan Hydrogels Combined with Different Types of Cells Aimed at Applications in Cartilage Regeneration

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Objectives: Injuries of the articular cartilage are one of the most challenging issues of musculoskeletal medicine due to the poor ability of this tissue for repair. Cartilage tissue engineering strategies require the presence of cells and a scaffold material, typically a hydrogel. In this work we have analysed the in vitro performance of k-carrageenan, an ionic hydrogel recently proposed for TE approaches, with encapsulated cells of different types, namely a chondrocytic cell line, primary chondrocytes cells and human adipose stem cells, often proposed for cartilage regeneration strategies. **Methods:** The kcarrageenan hydrogels were produced using an ionotropic gelation method and cells,

namely ATDC5 cells, human nasal primary chondrocytes (hNCs) and human adipose stem cells (hASCs), were encapsulated at a density of $5*10^6$cell/ml and cultured for 21 days. The cells viability and proliferation was determined by fluorescence staining, DNA quantification. Chondrogenic differentiation of the different cells encapsulated in the hydrogels was characterized by GAGs quantification, typical histological staining and real time qRT-PCR analysis (Sox9, aggrecan collagen type I, type II and type X). **Results:** The biological evaluation of k-carrageenan hydrogel revealed that this polymer enables long term viability and proliferation of different cells. During 3 weeks of culture, cells encapsulated within the hydrogel developed a cartilage-like extracellular matrix rich in proteoglycans and type II collagen. Cartilage-like ECM deposition and production was found throughout all culturing periods indicating a stable chondrocyte phenotype in encapsulated cells. Nevertheless, encapsulated hASCs exhibit the highest proliferation rates and highest levels of chondrogenic markers expression. **Conclusions:** K-carrageenan hydrogels enable the viability and proliferation of different cell types during long term cell culture. The results obtained indicated the feasibility of using these hydrogels in cartilage tissue engineering approaches due to its ability to support chondrogenic features of different cells types, particularly the hASCs.

P142 (EI0368)

Novel Gellan Gum—Hyaluronan Hydrogel Formulations for Tissue Engineering Applications

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Objectives: The main goal of this work consisted on the preparation and optimization of glycosaminoglycans (GAGs) hydrogels constituted of hyaluronic acid (HA) and gellan gum (GG) to support cell encapsulation to be used in tissue engineering (TE) purposes. **Methods:** Different GG-HA hydrogels formulations, ranging from 1 to 2.5% (m/V) of GG (1 MDa) and from 0.25 to 1% (m/V) of HA (3.5 KDa and/or 2 MDa) were prepared. The *in vitro* enzymatic degradation was evaluated by incubating the hydrogels with hyaluronidase solutions (0, 2.6 and 50 U/ml) for quantification of the resultant fragments using the Morgan-Elson and DNS assays. The mechanical properties of the developed hydrogels were determined by compression tests. The crosslinking efficiency was confirmed by 1H NMR and FTIR-ATR. Finally, the hydrogel morphology was visualized by SEM and micro-computed tomography (microCT). The best formulations were selected for further biological assays. Indeed, hASCs were encapsulated in the different hydrogels while the polymerization process occurred. The viability of the encapsulated hASCs was followed along 3, 7 and 14 days after Calcein-AM and Propidium Iodide staining. Cell morphology was visualized after phalloidin staining. **Results:** Hydrogels with different mechanical properties were obtained by altering the % (m/V) of the GG-HA formulations. Hydrogels with high percentage of GG were stiffer, while increasing concentrations of HA promoted hydrogel flexibility and higher degradation rates. Moreover, the hydrogels showed an intermediate degradation rate compared to the currently used photocrosslinkable HA-methacrylated hydrogels that rapidly degrade in PBS at 37°C. Furthermore, crosslinking efficiency was confirmed by FTIR-ATR analysis. The hASCs viability was not compromised by the hydrogels. **Conclusions:** This work permitted to obtain innovative GG-HA based hydrogels with tuned properties according to the different compositions. More importantly, their capacity to support cell encapsulation makes them very appealing for different TE applications.

P143 (EI0367)

Cryopreservation of Engineered Tissues: CT-Visualization of CPA Diffusion in Scaffolds

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Objectives: Effective long-term storage of native and engineered tissues poses a specific challenge for biomedical applications. The diffusion of cryoprotective agents (CPA) into tissue is one of the major hurdles for successful cryopreservation. In 3D engineered scaffolds CPAs like dimethyl sulfoxide (DMSO) should be homogeneously distributed to protect cells from freezing damages. A local excess of CPA in the construct will damage the cells due to the general toxic effects of CPAs, whereas insufficient CPA concentrations will lead to cryopreservation damage. This study was performed to measure and visualize the effective diffusion of DMSO within engineered collagen scaffolds using computer tomography (CT). **Methods:** Collagen scaffolds with a pore size of 100 µm (dimension: $30 \times 30 \times 10$ mm^3) and a porosity of 98% were self-manufactured [2]. Unseeded scaffolds and with 3T3-NIH cells seeded scaffolds were stored in PBS and respectively DMEM. The scaffolds were transferred directly in 10% (v/v) DMSO. Computer tomographic images were acquired immediately every 1.5 minutes over a period of 3 hours. Grey scale values that were determined from the images were converted in DMSO concentration und indicates CPA dispersion within the tissue. The DMSO loading process of the scaffold could thus be measured and visualized in real time. **Results and conclusions:** The study showed that incubation times of more than 3 h are required to achieve homogenous CPA distribution in unseeded collagen scaffolds of this size [3]. A model, established from experimental data, allows to determine adequate exposure times for different construct sizes.

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P144 (EI0360)

Using Human Adipose-Derived Stem Cell on Bacterial Cellulose Membranes for the Production of New Skin Substitutes

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Objectives: The study of the dynamic behavior and adhesion between cells and substrates in skin tissue engineering is of major importance to predict the final biological properties of tissue implants. The adhesion of cells on the substrate influences morphology, proliferation and cellular viability. In this work, adhesion, proliferation and viability of s on bacterial cellulose (BC) membranes were evaluated *in vitro*. The objective of this work was to demonstrate a method for obtaining new skin substitutes using human adipose-derived stem cell and keratinocytes on bacterial cellulose membranes. **Methods:** Biological effects of these bacterial cellulose membranes were evaluated by transmission electron microscopy (SEM), confocal microscopy, protein expression, viability and cytokine assays. **Results:** All these results suggest that bacterial cellulose membrane is a potential candidate for dermal equivalent with enhanced biostability and good biocompatibility. **Conclusions:** These results indicate that h ASC seeded onto bacterial cellulose membranes allowed cell adhesion, growth, proliferation and viability. We believe that the present model for human skin reconstructed *in vitro* has excellent applicability in relation to laboratory studies and good prospects for clinical use.

P145 (EI0359)

New Approach to Human Adipose Stem Cell Seeded on PHB-HV Scaffolds for Bone Tissue Engineering Applications

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Objectives: Tissue engineering emerges as a field of regenerative medicine that offers a strategy to circumvent the major problems related with regenerative and therapeutic procedures including bone grafting. Therefore, our group have examined the possibility of using a pool of allogeneic human serum (alloHS) as supplement of the osteogenic medium to evaluate the growth patterns and osteogenic differentiation of hASC on poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHB-HV) scaffolds, aimed to be used in bone tissue engineering. **Methods:** In this study, hASC were obtained from lipoaspirates and expanded in medium containing Dulbecco's modified Eagle's medium/F12 supplemented with 10% alloHS. Immunophenotypic characterization with flow cytometry was performed. The PHB-HV scaffolds used were developed by freeze-drying technique and characterized by SEM and µCT. The cells were seeded onto the PHB-HV scaffolds under static condition and cultured by different times in nonosteogenic and osteogenic medium. Through *in vitro* assays, the viability of these cells was determined by MTS assay. Cell adhesion and morphology were observed by SEM. And the osteogenic potential of these hASC were assessed by alkaline phosphatase quantification, mineralization content and expression of bone markers. **Results:** The scaffolds showed a good porosity and interconnectivity allowing adhesion and proliferation of hASC. The cells cultured in DMEM 10% alloHS showed the immunophenotype characteristic for mesenchymal stem cell, high viability and were capable of differentiate into the osteogenic lineage. **Conclusions:** All these results indicate that hASC seeded onto PHB-HV scaffolds and cultured in alloHS medium

may be a suitable strategy to induce bone formation. And the data presented here are important for bone tissue engineering.

P146 (EI0357)

Dendritic Cells as Relevant Tools to Predict the Outcome of Tissue Engineering Constructs

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Objectives: Within TE constructs, cells represent adjuvants to the host immune system, acting through the maturation of dendritic cells (DCs), leading to increased co-stimulatory and MHC molecules expression, cytokine secretion and allo-stimulatory capacity. Starch and poly-caprolactone (SPCL) scaffolds have shown to support human adipose-derived mesenchymal stem cells (hASCs) growth and differentiation. The aim of this work was to evaluate the interaction of hASCs within SPCL scaffolds with DCs, gathering further knowledge on the immunomodulatory potential of these constructs. **Methods:** SPCL scaffolds, obtained by wet spinning methodology (SPCL-WS), seeded with hASCs were cultured in standard cell culture conditions for 48 h prior to contact directly with DCs for further 24 h, 48 h and 72 h. DCs were differentiated from the mononuclear fraction of human peripheral blood cultured with IL-4 and GM-CSF for 48 h. The maturate and activated phenotype of DCs was evaluated by flow cytometry and RT-PCR before and after culture with the TE constructs, and compared with mature DCs, expressing CD80, CD83, CD86 and MHC class-II and lacking CD14 after incubation with LPS. **Results:** The findings showed that DCs maintain their immature status at days 3 and 7 days after replating, demonstrating low expression of CD80 and CD83. As replating procedures were shown to be a critical step in the routine evaluation of TE constructs this is a critical issue to address. Although DCs do not express the maturation markers, their genetic profile showed the presence of CD80, CD83, CD86 and CD1a, indicating that cells are capable of expressing those markers after adequate stimuli. **Conclusions:** These TE constructs (SPCL-WS scaffolds and hASCs) showed the inability to induce allogenic DCs activation after direct contact culture. This supports the conclusion that the assembled TE constructs will be well tolerated by the host in an allogenic approach and might further indicate their immunomodulatory potential.

P147 (EI0353)

Microencapsulation of Cells in Alginate Beads Using High Voltage Electrospraying

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Objectives: Cell transplantation is currently being investigated for treatment of various disorders such as liver failure, diabetes, etc. Since the source of these cells is nonautologous, immunogenicity is a major concern. To get around this problem, one can encapsulate the cells in a hydrogel matrix such as alginate beads. Encapsulation of cells can be also used to protect the cells during transportation, cryopreservation etc. Here we describe a method for production of such cell-encapsulated alginate beads using high voltage to control the size of the beads. **Methods:** NIH-3T3 cells were incubated in a suspension of 1.5% Alginate solution for 2 hours. The solution was drawn into a syringe, whose nozzle was connected to a high voltage source. The flow rate was controlled using a precision pump. Drops formed via electrospraying were collected in a cross-linking bath of $CaCl_2$ or $BaCl_2$ which was kept grounded. The resulting beads were washed with PBS before determining postprocess cell survivability using fluorescence microscopy. **Results:** The typical size of the beads ranged between 800 µm and 1.3 mm, which could be controlled by changing the process parameters such as flow rate and the applied voltage. The number of cells encapsulated per bead could be controlled by changing the initial cell concentration in suspension. Qualitative analysis using fluorescence imaging showed that cells survived the process. **Conclusions:** Here, we describe a method to produce cell-encapsulated alginate beads using high voltage. Compared to gravity-driven systems, this technique offers more control over the size of the beads. Further experiments are currently being planned to study long-term effects of this process, as well as the possibility to encapsulate other cell types. This work is supported by funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for the Cluster of Excellence REBIRTH (From Regenerative Biology to Reconstructive Therapy).

P148 (EI0371)

Three-Dimensional (3D) Capillary-Like Structure Formation with the Help of Biofunctionalised Microfibers

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Objectives: Cell-providing vessels are indispensable throughout an engineered tissue with more than 100–200 µm of thickness. To control vascular network formation in-vitro, fibres can be used to position vessel-forming endothelial cells within a 3D matrix. This support can then lead to capillary-like structure formation next to the fibres, supported by growth factors. With this method, a local-controlled capillarisation can be achieved. **Methods:** Biofunctionalization of poly-DL-lactide acid (PDLLA) fibres is done by aminofunctionalization and covalent binding of RGD peptides, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) via a hexamethylene diisocyanate (HDI) spacer. RGD is an amino acid sequence supporting cell attachment, whereas VEGF and bFGF promote capillary-like structure formation. After positioning of the biofunctionalized fibres in a moulding form, human umbilical artery smooth muscle cells (HUASMCs) and human umbilical vein endothelial cells (HUVECs) were seeded on the fibres. Fibrin gels were moulded directly or 4 days after cell seeding on the fibres inside the wells. After 9 days of coculture, the gels were fixed and immunostained with CD31. The formation and quantification of capillary-like structures in the 3D fibrin matrix was done using two-photon microscopy and ImagePro Analyzer software. **Results:** Capillary-like networks formed mainly on the lowest plane of the fibrin gel, but also near the fibres. When the fibrin moulding was done 4 days after cell seeding, capillary-like structures formed directly next to the functionalized microfibers. The cocultivation of HUASMCs seem to sufficiently support HUVECs by forming capillary-like structures. **Conclusions:** Vascular network formation can be realised and controlled next to biofunctionalized fibres inside a 3D fibrin matrix. Two-photon microscopy helps to visualize and quantify the capillary-like structures. Ideally, degradable fibres should be used in the future while the intact capillary-like network remains inside the fibrin matrix. Furthermore, mechanical stimuli can help building up tubule-like structures with lumen.

P149 (EI0350)

Electro Hydro Dynamic (EHD) Encapsulation of Cells in Alginate Based Hydrogels

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Objectives: The possibility of cells encapsulation in a polymeric bead with proper characteristics offer many advantages for clinical applications in tissue repair since is possible to tailor these beads for a controlled release of entrapped cells on a repair site. The objective of this study is to evaluate the possibility to use an electro hydro dynamic (EHD) system as a mean to encapsulate living mammalian cells in alginate based hydrogels. **Methods:** Capsules are manufactured starting from a tailored alginate based liquid solution containing mammalian cells, drops are formed through a needle by a EHD system and these drops are immediately crosslinked in a calcium based solution. The effect of the process on cell viability is assessed and confocal microscopy images will be presented. **Results:** EHD seems a versatile technique to encapsulate living cells. It allows the creation with high throughput of round beads up to 100 um in which cells remain viable starting from solutions characterized by a wide range of viscosity. The viscosity of the solution has an impact on the ability of forming and detaching drops from the EHD needle and as such on their final geometry, while the crosslinker properties determine the morphological characteristics of the final capsules. **Conclusions:** If the encapsulation of a given cell line is desired it is important to know what are the ranges of the parameters that characterized the encapsulation process that can be used in order to obtain beads with desired geometry containing viable cells for a specific application.

P150 (EI0375)

Combinatorial Cell-Porous Scaffolds Interactions Study and Scaffold Physicochemical Characterization in an Innovative Bioinspired High-Throughput Platform

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Objectives: Superhydrophobic surfaces patterned with size-controlled hydrophilic spots are proposed for the production of porous scaffolds arrays for combinatorial biomaterial-cells interaction studies. This approach allows for open and easy access to scaffolds for further follow ups aimed at high-throughput screening, when compared with the other scaffold array production techniques using commercial well plates or soft lithography-produced wells. Mechanical and morphological characterization along with cell study using chitosan/alginate mixtures with different concentrations of fibronectin were the proposed objectives. **Methods:** Polystyrene superhydrophobic surfaces were prepared by a phase-separation method. Aluminum hollow masks were used to pattern 4 mm^2 hydrophilic spots by UV/O_3 exposure. Chitosan/alginate mixtures in 100:0, 75:25 and 50:50 proportions along with 3 different concentrations of fibronectin were placed on the spots. L929 and MC3T3 cell lines were used for cell viability (by MTS test) and cell number (by dsDNA quantification) study. Future work aims for the characterization of the structures on the chip by dynamic mechanical analysis and µCT. **Results:** Cell culture results were mostly favorable for 50:50 chitosan/alginate proportion for both cell types. In this condition, fibronectin in the highest concentration enhanced cell viability using L929 cells, while in MC3T3 cells the use of all concentrations of fibronectin showed increased adhered viable cells compared to the non-adsorbed fibronectin condition. Regarding cell number, generally, the presence of fibronectin affected MC3T3 cell line favorably, as well as L929 in the 100:0 polymer ratio condition. Overall, a tendency for enhanced cell response can be seen in the highest concentration of fibronectin. **Conclusions:** A biochip for combinatorial analysis of biomaterial-cells interactions based in the extreme wettability of patterned spots in superhydrophobic surfaces was developed for the study of natural polymer porous scaffolds. Tendencies could be seen in cell response of two cell lines according to polymer and adsorbed protein gradients.

P151 (EI0343)

L-Proline and Ectoine Stabilize Proteins from Denaturation

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Objectives: Compatible solutes such as ectoine, hydroxyectoine and L-proline have been reported to be able to stabilize lipids and proteins in cell membranes. Recently it was found that L-proline and ectoine could be applied in cryopreservation of human stem cells as cryoprotective agents (CPA). Thus the conventional CPA dimethyl sulfoxide which is cell toxic could be replaced. The working mechanism of compatible solutes was explained as "preferential exclusion." In this study we have studied effects of L-proline and ectoine on the denaturation of model protein bovine RNase A with differential scanning calorimeter (DSC). **Methods:** Nano DSC from TA Instruments was used in this study. Bovine RNase A which is well studied was used as model protein. Protein solution was mixed with different concentrations of L-proline and ectoine (between 10 mM and 4 M), end concentration of RNase A was kept at 2 mg/ml. Samples were examined with DSC in a temperature range from 20°C to 100°C. Protein denaturation onset temperature, melting temperature, calorimetric enthalpy, change of the specific heat capacity and the fraction of the denatured protein were determined by analyzing the DSC data curves. **Results:** DSC results showed that L-proline and ectoine elevate melting temperature with a positive relationship with their concentration, in the presence of 2 M L-proline, protein melting temperature was 1.5 K higher than that without L-proline. Melting enthalpy of bovine RNase A was also increased, small concentrations of L-proline (10 mM to 100 mM) could lead to the maximum elevation, however ectoine needs relative higher concentration (500 mM). In the presence of 2 M L-proline and 4 M ectoine, protein denaturation was highly. The results are in agreement with literature data. **Conclusions:** Compatible solutes L-proline and ectoine are able to stabilize bovine RNase A, L-proline needs relatively lower concentrations as compared to ectoine.

P152 (EI0382)

Dental Pulp Stem Cells and Nanofibrous Scaffolds for Application in Tissue Engineering

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Objectives: Organs of the human body are nanostructures and cells in the body are accustomed to interact with materials that have nanostructured features and hence development of nanofibers by using the technique of electrospinning is having a new momentum. Smart engineered scaffolds loaded with stem cells can differentiate into specific cell lineages for effective tissue regeneration. Regarding to importance of stem cells and nanofibrous scaffolds, the aim of this study is the investigation of suitability of Poly (ε-caprolactone) PCL/gelatin nanofibrous scaffolds for dental pulp stem cells attachment and proliferation. **Methods:** The polymer solution with concentration of 6 wt% of PCl/gelatin (70:30) was electrospun at high voltage of 12 kV. The morphology, mechanical properties and hydrophilicity of nanofibers were studied using scanning electron microscopy (SEM), uniaxial testing and contact angle measurement respectively. Dental pulp stem cells were seeded on PCL/gelatin nanofibrous scaffolds and tissue culture plate (TCP) as control. MTS assay was used for investigation of proliferation of stem cells on nanofibrous scaffolds after 3 and 5 days of cell seeding. The morphology of cells on nanofibrous scaffolds was observed after 5 days of cell seeding using SEM. **Results:** From SEM images of nanofibers, fiber diameter was estimated to be 189 ± 56 and mechanical testing and contact angle measurement revealed the suitability of PCL/gelatin nanofibrous scaffolds in sense of tensile strength and hydrophilicity. MTS assay revealed the proliferation of stem cells on PCL/gelatin nanofibrous scaffolds. The cell proliferation on nanofibers was found to be higher than that on TCP after 5 day of cell seeding. SEM micrographs of cells on PCL/gelatin nanofibers also showed the attachment and spreading of cells on nanofibrous scaffolds. **Conclusions:** In summary, our results showed the suitability of PCL/gelatin (70:30) nanofibrous scaffolds as a substrate for dental pulp stem cells.

P153 (EI0384)

Dental Pulp Stem Cells and Nanofibrous Scaffolds for Application in Tissue Engineering

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Objectives: Organs of the human body are nanostructures and cells in the body are accustomed to interact with materials that have nanostructured features and hence development of nanofibers by using the technique of electrospinning is having a new momentum. Smart engineered scaffolds loaded with stem cells can differentiate into specific cell lineages for effective tissue regeneration. Regarding to importance of stem cells and nanofibrous scaffolds, the aim of this study is the investigation of suitability of poly (ε-caprolactone) PCL/gelatin nanofibrous scaffolds for dental pulp stem cells attachment and proliferation. **Methods:** The polymer solution with concentration of 6 wt% of PCl/gelatin (70:30) was electrospun at high voltage of 12 kV. The morphology, mechanical properties and hydrophilicity of nanofibers were studied using scanning electron microscopy (SEM), uniaxial testing and contact angle measurement respectively. Dental pulp stem cells were seeded on PCL/gelatin nanofibrous scaffolds and tissue culture plate (TCP) as control. MTS assay was used for investigation of proliferation of stem cells on nanofibrous scaffolds after 3 and 5 days of cell seeding. The morphology of cells on nanofibrous scaffolds was observed after 5 days of cell seeding using SEM. **Results:** From SEM images of nanofibers, fiber diameter was estimated to be 189 ± 56 and mechanical testing and contact angle measurement revealed the suitability of PCL/gelatin nanofibrous scaffolds in sense of tensile strength and hydrophilicity. MTS assay revealed the proliferation of stem cells on PCL/gelatin nanofibrous scaffolds. The cell proliferation on nanofibers was found to be higher than that on TCP after 5 day of cell seeding. SEM micrographs of cells on PCL/gelatin nanofibers also showed the attachment and spreading of cells on nanofibrous scaffolds. **Conclusions:** In summary, our results showed the suitability of PCL/gelatin (70:30) nanofibrous scaffolds as a substrate for dental pulp stem cells.

P154 (EI0385)

Artificial Let-7g Downregulation for Induction of Liver Regeneration—Initial Results with HepG2 Cells and Primary Human Hepatocytes

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Objectives: It is already known that global down-regulation of microRNA family let-7 members plays a crucial role in carcinogenesis due to their function as growth suppressor. Recent data indicate that temporal let-7 downregulation is involved in regulation of liver regeneration. The aim of this study was to investigate if artificial downregulation of let-7g could be used to induce up-regulation of proliferation-inducing genes in tumor cells and quiescent primary human hepatocytes (PHH). **Methods:** HepG2 cells and PHH were used for in vitro studies. Antisense oligonucleotides against hsa-let-7g were inserted by liposom transfection for 24 hours. Effects of transfection were investigated using real-time Polymerase Chain Reaction. Protein expression of putative targets of let-7 family members (Cyclin D1 and c-Myc) was investigated by Western blot analysis. **Results:** Endogenous expression of let-7g was lower in HepG2 cells compared to PHH. Transfection of HepG2 and PHH with anti-sense-let-7g caused significant decrease of endogenous let-7g expression. We reached a knockdown ratio of nearly 100% in cultivated HepG2 and in PHH. While we did not observe alterations in protein expression of c-Myc and Cyclin D1 in HepG2-cells, we observed significant upregulation in PHH following artificial let-7g knockdown. **Conclusions:** Our initial results show that a depletion of let-7g seems to have an increasing effect on cell cycle modulating proteins such as c-myc and Cyclin D1 in cultivated PHH. The down-regulation of let-7g could be a potential opportunity of miRNAs based therapeutical strategies for induction of liver regeneration.

P155 (EI0331)

Rational Design for Irrigation Dripping Tripled Perfusion Bioreactor

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Objectives: The postprinting tissue maturation requires development of new type of perfusion bioreactor. The rational design behind such bioreactor, especially the level of porosity and distance between minitubes must be based on systematic mathematical modeling and computer simulation of interstitial flow. The proposed novel irrigation dripping tripled perfusion bioreactor will enable to "buy" time until the "built in" intraorgan branched vascular system will mature enough for initiation of intravascular perfusion. **Methods:** Fick formula has been used to estimation diffusion coefficient for oxygen and small molecular weight tracers such as dextran or bovine serum albumin. It was assumed that hydrogel has isotropic properties. Mathematical model for diffusion and diffusion enhanced by convection has been developed. Computer simulation of diffusion gradient has been explored using color coding of trace molecule concentration. **Results:** Mathematical modeling has shown that with increasing minitube porosity the diffusion distance will also increase. The diffusion with additional convection will increase distance between porous minitubes. Thus, rational design of irrigation dripping tripled perfusion bioreactor must have combination of proper level of minitube porosity and maximal possible and biologically acceptable distance between minitubes. Computer simulation of interstitial flow and estimated parameters for rational design of perfusion bioreactor have been confirmed experimentally by analysis of diffusion of tracer molecules from porous needle placed in different type of hydrogels. It have been also demonstrated that during tissue maturation the diffusion and interstitial flow in bioprinted tissue construct will be gradually reduced. **Conclusions:** Mathematical modeling and computer simulation have been used to estimate proper design parameters. Thus, it has been demonstrated that with implementation of rational design based on mathematical modeling and computer simulation development of irrigation dripping tripled perfusion bioreactor is a realistic goal.

P156 (EI0329)

Scalable Biofabrication of Uniformed Sized Tissue Spheroids for Organ Printing

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Objectives: Organ printing is a rapidly emerging technology based on layer by layer additive robotic biofabrication of 3D tissue and organ constructs using self-assembling tissue spheroids as building blocks. In order to print human organ constructs it will be necessary to fabricate millions of tissue spheroids. In this context the development of methods for scalable biofabrication of uniformly sized tissue spheroids is essential for tissue spheroid-based bioprinting of large size tissue and organ constructs. **Methods:** Two new molds were designed to enable generation of microrecessions in nonadhesive agarose hydrogel. The microrecessions were seeded with human adipose tissue derived stem cells using manual and automated pipetting approach. Size redistribution and shape of biofabricated tissue spheroids have been estimated. **Results:** After 48 hours of incubation, tissue spheroids formed at the bottom of each microrecession. To assess the quality of constructs generated using this technology, six hundred tissue spheroids made by this method were compared with six hundred spheroids generated by the conventional hanging drop method. These analyses showed that tissue spheroids fabricated by the micromolded method are more uniform in diameter and shape than by conventional hanging drop methods. The main advantage of using new molds with optimized geometry is capacity to use robotic dispenser and thus automate and scale up process of more uniform tissue spheroids biofabrication. **Conclusions:** Thus, use of micromolded recessions in a nonadhesive hydrogel, combined with automated cell seeding, is a reliable method for scalable robotic biofabrication of uniform sized tissue spheroids. The tissue spheroids of standard size could be used in drug discovery and toxicity assays, in direct differentiation of stem cells into specific minitissues (for example cartilage or bones) and most importantly for robotic bioprinting of complex human organs.

P157 (EI0321)

Influence of Polylactide Fibers With Spin Finishes on the Toxicity in Vitro

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Objectives: Biodegradable fibers with controlled properties may meet the requirements for medical applications. Poly(lactic acid) (PLA) is a biodegradable linear aliphatic thermoplastic polyester. There were prepared PLA fibers with five type spin finishes which were assayed for in vitro cytotoxic activities. **Methods:** The PLA fibres were prepared by a two-step melt-spinning process. The PLA Polymer 6201D, fiber grade with nominal MFI = 15–30 g/10 min, a NatureWorks LLC product was used. PLA fibers were coated with 5 types of spin finishes: PLA 24 with 2.4% of Glicerol Ph Eur, PLA 25–0,40% of Lurol PL 801, PLA 26–0,61% of Stantex 6457, PLA 27–0,36% of Lurol PT-L216, PLA28- 0.62% of Estesol PF 790. The fibres with linear density 2,2–4,8 dtex, tenacity 35–39 cN/tex, elongation ~50 % were obtained. To determine if they can affect cells, line cultures L929 (ATCC CCL1) was used. The cells (2×10^6 cells/ml) were incubated with fibres for 24 h, 48 h and 72 h (37°C, 5% CO_2). Cell growth, morphology and viability were determined. **Results:** After 72 h incubation, the level of cytotoxicity of PLA 24 fibers was 2 (% dead-38), PLA 25-3 (% dead-100), PLA 27-3 (% dead-100), PLA 28-0 (% dead-99), control fenol-3 (% dead-94), L929-0 (% dead-3). **Conclusions:** Fibroblast cultures after contact with the four of PLA fibres showed cytotoxity effects. The cells were dead with hanged morphologie and lower proliferation. The result of the testing of PLA fibers with Estesol spin finish did not show any cytotoxicity effects and may be promising candidate for medical applications. *Financial support by the project "Biodegradable fibrous products", POIG.01.03.01-00-007-/08-00 and EU in the frame of IE OP financed from the ERDF, is gratefully acknowledged.*

P158 (EI0316)

PRP Influence on the Regeneration of Reproductive System in the Experiment

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Objectives: analysis of medical influence of platelet rich plasma (PRP) on the reproductive function of mice in experimental toxic influence condition. **Methods:** ICR mice at the age of 4–5 months were used. For modeling pathology of reproductive system 2 intraperitoneal injection of Adriblastin in summary dose of 2 mg/kg were done with an interval of one week. The mice were divided into two groups: the 1st is the control group; in the mice of the 2nd group we have injected PRP in scrotum on the second day after last injection of Adriblastin. PRP was injected three times with an interval of 2 weeks. Pathomorphologic analysis of sections was done on the 4 and 6 weeks after PRP injection. **Results:** On the fourth week the extension of convoluted channels, significant decreased amount of spermatogonia and other cell types of spermatogenic epithelium can be noticed in the 1st group in comparison with intact animals. Two weeks later interstitium and basal membrane are extended and hyperchromic, due to edema and inflammation, the amount of spermatozoa is still reduced. In the second group visible increase of all cell types of spermatogenic epithelium can be noticed after 4 weeks post treatment. Spermatogonia recovery is an evidence of high healing effect of PRP, because Adriblastin influence negatively exactly on these germinative cells of spermatogenic epithelium. Interstitium and basal membrane are still extended, but they are in better morphologic condition as they are in the first group in the meantime. On the 6th week convoluted channels are partially extended, but

they are reverting to the norm. The state of organ is recovering. **Conclusions:** PRP makes good results in treating of toxic defeat of reproductive system.

P159 (EI0315)

The Degradation of Porcine Endogenous Retroviruses DNA in Acellular Porcine Heart Valve Scaffolds Fixed with Different Low Toxic Agents

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Objectives: The application of tissue engineered scaffolds based on porcine heart valves is connected with danger of porcine endogenous retroviruses (PERVs) transmission because acellularization process might not remove all PERVs DNA from tissue. Chemical fixation with glutardaldehyde induces complete degradation PERVs genetic material in porcine tissue, but this compound is too toxic for cells which will be seeded on scaffolds. Therefore the purpose of the present study was to investigate how chemical fixation with low toxic agents i.e. two different derivatives of flavonoids—DF1 and DF2, and genipin influence on the PERVs DNA existence in acellular porcine heart valve scaffolds. **Methods:** Porcine pulmonary and aortic valves were acellularized using Trypsin/EDTA and sodium sulphate. Acellular tissues were treated with 1) flavonoid derivative DF1, 2) flavonoid derivative DF2 or 3) genipin. The fixation was carried out at 20°C for 3 days. Genomic DNA was isolated from native, acellular and acellular fixed tissues by means of salting out extraction method. Quantification of PERV-A, PERV-B and PERV-C DNA was performed by real time Q-PCR technique. Native and acellular valves were used as a control. **Results:** All subtypes of PERVs were detected in native porcine heart valves. Reduction of copies number of PERV-A, PERV-B and PERV-C DNA was observed in acellular porcine valves as well as in acellular valves fixed with flavonoid derivative DF1 and with genipin. PERVs DNA was completely degradated only in acellular porcine heart valves fixed with flavonoid derivative DF2. **Conclusion:** Our results demonstrated that fixation of acellular porcine valves with flavonoid derivative DF2 causes completely degradation of PERVs DNA in tissues, thus the acellular porcine heart valve scaffolds fixed with low toxic agents can be used for transplantation without risk of PERVs transmission.

P160 (EI0407)

Interaction of Chondrocytes with Electrospun Polymer Scaffolds Depending on the Fibre Orientation

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Objectives: Biocompatible polymer-based scaffolds with a tailaroble degradation rate and a predefined structure might provide an approach to improve cartilage repair, which is limited by the poor intrinsic healing capacity of cartilage. The aim of this study was to explore, whether electrospun polymer scaffolds with different fibre orientation could influence the growth of primary articular chondrocytes. **Methods:** Electrospun scaffolds with aligned and random fibre orientation were prepared from two degradable polymers: poly(ether)ester urethane (PDC) and poly(*p*-dioxanone) (PPDO) as well as polyetherimide (PEI) as a reference polymer, which is not intended to degrade. PDC was selected as a candidate material showing an almost linear mass loss in hydrolytic and enzymatic in-vitro degradation experiments. Electrospinning was conducted at ambient temperature using hexafluoro-2-propanol (HFP) as solvent for PDC and PPDO, while PEI was processed from dimethylacetamide (DMAc) solution. The electrospun structures exhibited an average deposit thickness of 80 ± 20 µm with a single fibre diameter around 2–3 µm. Primary porcine articular chondrocytes were seeded on the ethylene oxide sterilized scaffolds and analysed for vitality, ultrastructure and type II collagen expression. **Results:** Satisfactory numbers of vital chondrocytes could be detected on all electrospun scaffolds, which were able to produce the cartilage-specific protein type II collagen. An almost flattened cell shape of the chondrocytes was observed on scaffolds with random fibre orientation, while on scaffolds with aligned fibres the chondrocytes exhibited a spherically cell shape and penetrated into the scaffold pores between the parallel fibres. Surprisingly, it was found that the chondrocytes did not align along the fiber direction. **Conclusions:** Chondrocytes were able to grow on all polymer scaffolds tested and expressed the differentiation marker type II collagen. Cell morphology differed depending on the fibre orientation within the scaffolds.

P161 (EI0291)

Ionic Liquids in Novel Processing Ways to Obtain Chitosan/Silk Fibroin Hydrogels for Skin Tissue Engineering

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Objectives: The main goal of this work renders the application of green chemistry principles, namely the use of ionic liquids (ILs) and biorenewable sources, such as chitosan (CHT) and silk fibroin (SF), to newly process hydrogel-based constructs. The combination of this polysaccharide and protein may mimic the naturally occurring tissue environment. Although the solubilization of both materials in ILs has been studied individually, this work reports, for the first time, the use of ILs as a common solvent, for blended CHT/SF hydrogels production. These systems offer the advantage of being homogeneous and of presenting easy and short dissolution time of both biomacromolecules. Moreover, the intrinsic properties of these biopolymers are expected to accelerate the regeneration of chronic skin wounds. **Methods:** Hydrogels were obtained by dissolving CHT and SF in 1-butyl-3-methylimidazolium acetate, [bmim][Ac] (4wt%) at different ratios. The systems were gelified and ILs removal was performed by soxhlet extraction with ethanol. The effect of the chitosan source and CHT/SF ratio on consistency, crystallinity, protein adsorption and mechanical properties was evaluated. Moreover, the ability of the developed materials to support adhesion and proliferation of human dermal fibroblasts (hDFb) was assessed up to 21 days of culture. **Results:** The findings suggest that [bmim][Ac] allowed the production of CTH/SF hydrogels with a soft and rubbery consistency, microporous surface, good protein adsorption and viscoelastic behavior. Additionally, *in vitro* biological performance revealed a positive influence over adhesion, viability and proliferation of hDFb. **Conclusions:** The use of [bmim][Ac] as a common solvent provided a versatile approach to obtain CHT/SF hydrogels with interesting properties and with potential to sustain dermal fibroblasts outgrowth. This work constitutes a strong basis for future healing studies of chronic skin wounds.

P162 (EI0279)

Microvesicles Derived from Human Adult Mesenchymal Stem Cells Protect Against Ischemia-Reperfusion-Induced Acute and Chronic Kidney Injury

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Objectives: Several studies demonstrated that mesenchymal stem cells (MSCs) reverses acute kidney injury (AKI) in different experimental models by a paracrine mechanisms rather then by MSC transdifferentiation. We recently demonstrated that microvesicles (MVs) released from MSCs may account for this paracrine mechanism by an horizontal transfer of mRNA and microRNA. **Methods:** MVs were purified from MSC supernatants and were injected intravenously in rats (30 mg/rat) immediately after monolateral nephrectomy and renal artery and vein occlusion for 45 minutes. To evaluate the MV effects on AKI induce by IRI, the animals were divided into different groups: normal rats (n = 4), sham operated rats (n = 6), IRI rats (n = 6), IRI + MV (n = 6), IRI+ RNase-MV (n = 6) and all animals were sacrificed at day 2 after operation. To evaluate the CKD induced by IRI, the rats were divided into different groups: sham operated rats (n = 6), IRI rats (n = 6), IRI + MV (n = 6) and all animal were sacrificed 6 months after the operation. **Results:** We found that a single administration of MVs, immediately after induction of ischemia-reperfusion injury, protects rats from AKI by inhibiting apoptosis and stimulating tubular epithelial cell proliferation. The MVs also significantly reduced the impairment of renal function induced by ischemia reperfusion injury. Pre-treatment of MVs with RNase to inactivate their RNA cargo, abrogated these protective effects. Moreover, MVs protected from chronic kidney disease observed at 6 months in control rats with ischemia reperfusion injury. **Conclusions:** MVs released from MSCs protect from ischemia reperfusion induced AKI and chronic renal injury, suggesting that MVs could be exploited as a potential new therapeutic approach.

P163 (EI0278)

Fast Dynamic MRI Monitoring During Liver Cell Transplantation to the Spleen in a Porcine Model

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Objectives: Liver cell transplantation (LCT) is a promising approach for the treatment of metabolic liver disorders. However, a method for noninvasive monitoring during LCT is not available clinically, and thus little is known about the processes during and following LCT. The aim of this study was to investigate the feasibility of fast dynamic magnetic resonance imaging (MRI) monitoring during liver cell infusion to the spleen, which is considered an ectopic implantation site for LCT. **Methods:** Male porcine liver cells were labeled with micron-sized iron oxide particles (MPIO) and infused to the spleen of female fully-grown pigs (n = 5) through a catheter placed in the lineal artery. MRI monitoring was performed using a conventional 3.0 Tesla MR scanner. Initially, T1- and T2-weighted pulse sequences were tested for the detection of MPIO-labeled cells in the spleen. Thereafter, fast dynamic MRI was performed during cell infusion. MR findings were verified by histological and immunohistological examination. **Results:** Images from static MRI (repetition time / echo time: 2,500/105.2 ms) showed significantly lower signal intensityand signal-to-noise ratio after cell infusion compared to pretransplant images. T2-weighted fast dynamic MRI enabled visualization of continuous signal decrease of the spleen during cell infusion. T1-weighted sequences did not show signal decrease at the same time. When cells were infused systematically, no signal changes in the spleen were observed. **Conclusions:** This study shows that fast dynamic MRI can enable noninvasive visualization of liver cell distribution in the spleen and verification of the success of cell delivery. This approach could be useful for preclinical studies and for quality control of LCT in the clinical setting.

P164 (EI0267)

Silicon Grafted Collagen as a Scaffold for Repairing of Tympanic Membrane Perforations: in Vitro and in Vivo Assays

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Objectives: Chronic tympanic membrane perforations are treated by myringoplasty operation. Although multiple autologous grafts, allografts, and synthetic graft materials have been used over the years, no single graft material is superior for repairing all perforation types. Recently, our group have observed remarkable properties of collagen grafted polydimethyl siloxane (Col-g-PDMS) therefore, in this study Col-g-PDMS potential was assayed as tympanic membrane patch. **Methods:** Collagen was grafted onto substrate via a two-step plasma treatment. Then both the biocompatibility of the modified films and cell behaviour on the surface of these films were investigated by in vitro tests using mouse fibroblast cell (L929), and 12 patients underwent Col-g-PDMS myringoplasty in order to investigate its positional in vivo. **Results:** It was observed that collagen immobilized surfaces showed significant cell adhesion and growth in comparison with the unmodified samples. The overall efficacy of Col-g-PDMS myringoplasty was 75% with total closure, and reduction in size of perforation in 25% (after one attempt). In each of remaining three, there was a disturbing cause leading to the failure. **Conclusions:** The Col-g-PDMS grafts were found to be feasible for tympanic membrane perforations.

P165 (EI0265)

Influence of Conditioned Media in Cartilage-Like Tissue Production in Co-Cultures of Articular Chondrocytes and Wharton's Jelly Derived Stem Cells

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Objectives: Soluble factors released by chondrocytes have been shown to influence stem cells differentiation onto the chondrogenic lineage. The use of conditioned medium obtained from chondrocytes for stimulating stem cells chondrogenic differentiation may be a very interesting alternative for the clinical application of these cells. Therefore, we tested the influence of conditioned medium obtained from articular chondrocytes cultures to determine its influence on cocultures of human Wharton's jelly MSCs (hWJSCs). **Methods:** In the present work, direct and indirect cocultures (using conditioned medium obtained from a culture of human articular chondrocytes) with hWJSCs were established. Cells were isolated from human samples collected at the local hospital, under donors' informed consent. The cocultures were performed in 3D scaffolds, composed by a blend of 50/50 chitosan and poly (butylene succinate)—CPBS. Cocultures were maintained during 28 days. **Results:** Wharton's jelly MSCs were able to undergo chondrogenic differentiation. By the end of the experiment cocultures showed glycosaminoglycans (GAGs) accumulation and specific cartilage-related genes expression, for both types of cocultures. Indirect cocultures results show that the chondrogenic differentiation and cartilage ECM formation is enhanced compared to the direct ones. **Conclusions:** The use of conditioned medium obtained from articular chondrocytes induced WJSCs chondrogenic differentiation and ECM formation. The obtained results showed that this new strategy is very interesting and should be further explored for clinical applications.

P166 (EI0146)

The Synergistic Effects of Starling Flows and a Distributed and Delocalized Nutrient Source on Bone Marrow Stromal Cell Culture in Hollow Fibre Membrane Bioreactors

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Objectives: The development of large dimensions bone tissue engineered constructs is limited by the difficult delivery of nutrients to cells situated in the inner part of them. In this study, hollow fibre membrane bioreactors(HFMB) were used to allow a distributed and delocalized nutrients supply in 3D cm-scale constructs. Low to high spontaneus convective flows effect on cell distribution, proliferation and collagen deposition were investigated. **Methods:** HFMB where seeded with 4.5×10^6 BMSC cells/ml. Cells were fed with culture medium from the membranes lumen in a recirculation modality for 12 days. Bioreactors were operated to establish low to high convective flows towards the ECS. At the end of the culture cells were processed for scanning electron microscopy(SEM). Histological sections were stained with H&E to evidence cell radial organization and with DAPI to analyze cell nuclei distribution along the bioreactor length. Osteoblast phenotype maintenance was confirmed by pro-collagen immunostaining, collagen deposition detected with Masson–Goldner stain. **Results:** HFMB operated under low convective flows presented a uniform axial cell distribution and poor cell proliferation. Cells were found at the membrane surface forming a thin layer around them. SEM analysis confirmed the low proliferation rate under this operating condition. Histological analysis showed how in the presence of high spontaneous convective flows cells were dragged at the bioreactor outlet. Despite this evidence, this operating condition seems to be the most promising in obtaining large bone tissue engineered substitutes as demonstrated by the rearrangement of ostegenic cells in aggregates 2,5 cm thick. SEM analysis showed how cell number grew significantly respect to the seeding density forming a continuous thick multilayer covering the surface of several adjacent membranes. **Conclusions:** A delocalized and distributed nutrients supply coupled to bioreactor design promoting the occurrence of high Starling flows in the cell compartment may contribute to obtain clinical-scale 3D tissue engineered constructs.

P167 (EI0147)

Potential of Mesenchymal Stromal Cells Derived from Human Adult Adipose Tissue and Bone Marrow for Regenerative Medicine and Tissue Engineering

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Objectives: The aim of this study was to assess the clonogenic efficiency, immunophenotype and the potential of adipose tissue (AT) and bone marrow (BM) mesenchymal stromal cells (MSC) for *in vitro* differentiation into different mesenchymal and nonmesenchymal lineages. **Methods:** Human AT and BM were obtained with the patient informed consent under full Ethical guidelines. Immunophenotype of cells was determined by flow cytometry. The efficiency of adipogenic and osteogenic differentiation of cells was determined *in vitro* in media, supplemented with specific induction factors. Endothelial potential of MSC at different stages of culture was assessed using Matrigel assay. Differentiation of MSC into insulin-producing cells was prepared during culture in high glucose medium in the presence of pancreatic stimuli. **Results:** It was found that clonogenic efficiency of AT and BM derived MSC at passage 1 comprised 8,1 ± 1,8% and 8,5 ± 2,3%, correspondingly. The analysis of the adipogenic differentiation efficiency showed no significant differences in the percentage of differentiated cells as well as in triglycerides content between two MSC sources. The accumulation of extracellular calcium was 1,23 times higher in BM MSC cultures compared to AT MSC after osteogenic differentiation. Additional clonal analysis of AT MSC showed that about 50% of clones have the ability for osteogenic differentiation. MSC at different stages of cultivation were able to form capillary-like structures in the Matrigel confirming

the endothelial differentiation of cells. Differentiation of AT MSC and BM MSC into insulin-producing cells resulted in the formation of cluster-like aggregates and insulin expression, confirmed by PCR, ELISA and immunocytochemistry. After seeding into macroporous alginate-gelatin scaffolds, MSC from both sources demonstrated capability to proliferation and multilineage differentiation. **Conclusions:** MSC, derived from both BM and AT are promising for regenerative medicine and tissue engineering applications; however each of them has advantages and drawbacks for a special purpose, that will be additionally discussed.

P168 (EI0204)

Effect of Compressive Plane Strain on Osteoblast-Like Cells in Vitro

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Objectives: The aim of this research was to verify that a novel bioreactor was able to exert compressive stimulation on cells. Osteoblast-like cells were chosen as their normal environment is compressive and the cells themselves are not contractile. The bioreactor developed in this research is intended to give a useful and repeatable means to investigate the effect of compressive strain on the mechanobiology of osteoblasts. **Methods:** A novel bioreactor was used to apply cyclic compressive mechanical strain to cultured MG63 human osteosarcoma cells. Cells were seeded onto flexible polyurethane membranes and maintained in static culture in DMEM for 48 hours. The cells were then cultured for a further 48 hours while being stimulated by intermittent cyclic strain (1 hour at 30 cycles per minute), with strain magnitudes of up to 2%, then allowed 24 hours before fixation for imaging or protein analysis. At the conclusion of the experiment, cells were imaged using phase contrast microscopy and scanning electron microscopy, and protein expression was examined by PCR. **Results:** The results confirm the prediction by the classical theory that the concave face of a cantilever beam will be in compression. The effects of cyclic tensile and compressive strain on cell morphology, cell metabolism and protein expression are presented. Higher strain magnitudes were found to result in apoptosis, in accordance with the literature. **Conclusions:** The bioreactor developed in this research is able to exert compressive stimulation at physiological magnitudes on the cells studied. Stimulation of cells in conditions similar to those encountered *in vivo* will enhance our understanding of the mechanobiology of mammalian cells.

P169 (EI0081)

Preconditioning of Adipose Tissue-Derived Mesenchymal Stem Cells With Natural Molecules for Vascular Cell Therapy

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Objectives: Peripheral arterial disease represents a major health problem in modern society. If peripheral vascular occlusion progresses to ulceration or gangrene, the risk of limb loss becomes substantial. In patients where no medical treatment is considered effective for rest pain or ulcer healing, cell-based therapeutic angiogenesis has become a new promising hope. It has been reported that adipose tissue contains progenitors with angiogenic potential and that therapy based on adipose-derived mesenchymal stem cells (ADMSCs) administration can improve perfusion recovery in hindlimb ischemia mouse model. Here we aimed to enhance and optimize both vascular and perivascular commitment and paracrine patterning of human ADMSCs using natural molecules *in vitro*. **Methods:** ADMSCs were isolated from lipoaspirates, characterized, and treated with hyaluronic (HA), butyric (BU), and retinoic (RA) acid. Vasculogenic genes expression, including VEGF, KDR, HGF, and HIF-1α was analysed by Real-time PCR at 1-2-3-6 days. The presence of vascular (vWF) and perivascular (NG2, a-SMA, CD146, PDGF-Rβ) markers was evaluated by immunofluorescence and flow cytometry at 14 days. Secretion of angiogenic cytokines (VEGF, HGF) was assessed by ELISA. **Results:** Combinatorial treatment with HA, BU, and RA significantly increased the transcription of vasculogenic genes at every time point, and induced vWF expression, that is not detectable in untreated cells. The treatment remarkably augmented the percentage of cells expressing perivascular markers, and enhanced the secretion of angiogenic factors compared to control cells. Expression of PDGF-Rb, involved in proliferation of undifferentiated cells, is dramatically reduced in HA-BU-RA treated cells, suggesting a cellular commitment. **Conclusions:** ADMSCs represent an attractive alternative source of pluripotent cells compared to bone marrow in terms of accessibility and available tissue amount. The availability of natural molecules to enhance the endothelial and perivascular commitment of these cells may constitute a promising therapeutic approach for cell therapy in patients with ischemic vascular diseases.

P170 (EI0097)

Regeneration of the Intervertebral Disc (IVD) Using in Vitro Differentiated Stem Cells

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Objectives: The degeneration of the nucleus pulposus (NP) is one major reason for low back pain. One possible method of treatment is cell-based therapy with differentiated human mesenchymal stem cells (hMSC). For a differentiation into NP cells, the hMSCs require a 3D environment and various stimuli such as growth factors. In our work, we want to identify the optimal stimulation for the differentiation of hMSCs into NP cells. Regarding the therapeutically use it is indispensable to verify the differentiation success of hMSCs into NP cells and to delimit the differentiated cells to chondrocytes. As Raman spectroscopy has a high potential for noninvasive characterization of suspension cells and distinction between different cell lines, our research group together with the Fraunhofer Institute of Interfacial Engineering and Biotechnology (IGB) tested the applicability of this method for cells embedded in hydrogels. **Methods:** hMSCs were cultivated three-dimensional to form NP cells. To investigate the putative differentiation stimulating ability of several growth factors and components of the extracellular matrix (ECM), RT-PCR as well as fluorescence immunostaining of NP-specific marker proteins were done. **Results:** In all differentiation experiments with growth factors, NP-specific marker proteins were expressed. Data concerning the differentiation of hMSC under the influence of ECM components will be presented. Using Raman Spectroscopy as well as common methods of molecular biology, we were able to distinguish NP cells and differentiated hMSC from undifferentiated stem cells and chondrocytes. **Conclusions:** The expression of NP-specific marker proteins indicates the ability of three growth factors to differentiate hMSCs into NP-like cells. Using Raman spectroscopy and RT-PCR we could clearly display that NP cells differ from chondrocytes. As only pure NP cells (e.g. into NP cells differentiated hMSCs) could be used for subsequent therapeutically use, this finding is of great benefit for NP regeneration approaches.

P171 (EI0077)

Modulation of Angiogenesis by Fibroblasts in Tissue Regeneration

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Objectives: Promoting angiogenesis in a damaged tissue is a major challenge for tissue regeneration. It is important to observe and understand the morphology and the ability of cells to adapt to new biomaterial systems. The immobilization of cocultured bone progenitor cells with ECs within RGD-alginate has shown promising results as a bone regeneration strategy. In the present work, it is hypothesized that fibroblasts influence ECs and can thus improve angiogenesis. **Methods:** *In vitro* studies were carried out by investigating the influence of fibroblasts immobilized in a RGD-alginate matrix on EC assembly into capillary-like structures. An *in vivo* Matrigel plug assay was used and Hemoglobin levels and inflammatory factors were determined. **Results and Discussion:** *In vitro* studies showed that the presence of fibroblasts supported capillary-like structures formation for longer periods than controls (without fibroblasts). The length of the capillary-like structures were longer in the presence of immobilized fibroblasts compared to control conditions. *In vivo* studies using Matrigel plugs demonstrated that the presence of fibroblasts increased hemoglobin levels, although no significant differences were observed concerning N-acetylglucosaminidase activity and nitric oxide production, compared to controls (without fibroblasts). **Conclusions:** The present findings indicate that fibroblasts improved angiogenesis and did not seem to influence the inflammatory process in serum. Fibroblasts immobilized in RGD-alginate maintained their capacity to enhance angiogenesis. These findings provide evidence for the potential use of this strategy in tissue regeneration where vascularization is essential.

P172 (EI0090)

Oral Skeletal Tissue as an Applicable Source of Progenitor Cells for Regenerative Medicine

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Objectives: Tissue engineering is a promising approach for regenerative procedures in oral and maxillofacial surgery. This study investigated the suitability of oral skeletal tissue as an applicable source of progenitor cells and an alterna-

tive to the iliac crest bone marrow. The aim was to compare multilineage differentiation potential of osteoprogenitor cells and bone marrow mesenchymal stem cells (BM-MSCs). **Methods:** Osteoprogenitor cells were isolated from explant cultures of intra-orally harvested bone chips during routine oral surgery. BM-MSCs were obtained from iliac crest bone marrow aspirates and used as positive control for multilineage differentiation analysis. Cells were immunocytochemically characterized by the expression of characteristic surface antigens including CD73, CD90, CD105 and the lack of CD14, CD34, CD45. Differentiation capacities into the osteogenic, adipogenic and chondrogenic lineages were investigated using cytochemical tests (alkaline phosphatase activity, Oil Red O and Alcian blue staining) and RT-PCR analysis. **Results:** Osteoprogenitor cells showed characteristics of BM-MSCs like plastic adherence and expression of defined surface antigens. Their differentiation capacity into the osteogenic, adipogenic and chondrogenic lineages was comparable to the one of BM-MSCs. **Conclusions:** These findings suggest that osteoprogenitor cells have a similar differentiation potential to BM-MSCs' in vitro. Oral skeletal tissue may be considered as a suitable source of cells for tissue engineering therapies in regenerative dentistry.

P173 (EI0067)

The Reverse Remodeling Effect of Bone-Marrow-Derived Stem Cells Is Independent From the Site of Epimyocardial Cell Transplantation

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Objectives: The transplantation of bone-marrow derived stem cells represents a promising therapy in chronic heart failure. Positive effects of transplanting these cells could be shown, but the exact mechanisms are unknown. As paracrine effects are increasingly discussed, we evaluated if the injection site effects the amelioration on LV-contractility and angiogenesis in doxorubicin-induced failing hearts. **Methods:** Heart failure was induced in white New Zealand rabbits by doxorubicin (3 mg/kg for 6 weeks), followed by right-ventricular-transplantation (RV-BMC, n = 6), left-ventricular-transplantation (LV-BMC, n = 6), sham treatment (medium-group, n = 6), or no therapy (DOX, n = 5). Healthy rabbits were used as controls (control-group, n = 8). Cells were isolated by bone marrow aspiration and transplanted locally in the ventricle. Four weeks later cardiac function was assessed, and capillary density (CD31-staining) was measured. **Results:** The ejection fraction was significant higher in both BMC-groups vs. medium-group (LV-BMC 39.0 ± 1.4% vs. medium-group 29.8 ± 3.7% $p < 0.001$, RV-BMC 39.2 ± 2.6% vs. medium-group 29.8 ± 3.7% $p =< 0.001$), without significance between the BMC-groups ($p = 0.858$). The capillary density (capillaries/high-power-field) increased in both BMC-groups in all chambers of the heart compared to medium group. The left ventricular injection of BMCs increased the capillary density by 8.3 ± 3.4%, the right ventricular injection by 8.1 ± 2.2% compared to medium group without significant difference between the two BMC-groups. **Conclusions:** The beneficial effects of BMC transplantation in doxorubicin-induced cardiomyopathy are independent of the injection site. As BMCs failed to transdifferentiate into cardiomyocytes, paracrine factors seem to be responsible for the beneficial effects of stem cell transplantation.

P174 (EI0070)

Creatinine Transperitoneal Transport in Vitro—Influence of P-Cresol and Methylglyoxal

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Objectives: Functional and morphological modifications of peritoneum has been observed during long-term dialysis, peritonitis and cancer. Creatinine transfer is used as a marker to estimation of changes in peritoneal membrane. P-cresol can be consider to be compound with several toxic and some beneficial properties connected e.g. with protective antioxidative effects. Similarly, methylglyoxal is well-known reactive carbonyl solute with probably antiviral and anticancer properties. **Methods:** The object of study were analyses of p-cresol and methylglyoxal influence on creatinine transport across peritoneum in vitro. Membrane isolated from anterior abdominal wall of white New–Zealand rabbits, modified Ussing-type chamber and mathematical model of mass transfer were used to calculate the diffusive permeability coefficient P[cm/s] in the case of transport directed from the interstitial (I) to the mesothelial (M) side of the membrane and in the opposite direction. Four separate series of the experiments were done. In first and second one (control conditions) we examined the rates of the creatinine transport in the concentration gradients: 0.1 g/dL and 0.01 g/dL, respectively, during 120 minutes. In the next—values of P for creatinine (0.1 g/dL) before (15–60 minutes) and after p-cresol (0.005 g/dL) introduction into the experimental system (75–120 min) were investigated. In the fourth series—transfer of creatinine (0.01 g/dL) before (15–60 min) and after methylglyoxal (0.01 g/dL) applications (75–120 minutes) was analyzed. **Results:** Dynamics of the creatinine transport in both concentration gradients remained constant. The values of P+/-standard error of the mean were 2.340+/–0.265[×0.0001;cm/s] and 2.381+/–0.244 [×0.0001;cm/s] for I->M and M->I, respectively. Introduction of p-cresol into the experimental system did not alter the transfer of creatinine, both in the case of I->M and M->I passage. In contrast, methylglyoxal caused a 7% ($p < 0.01$) decline of the bidirectional transport of examined solute. **Conclusions:** In vitro methylglyoxal, but not p-cresol modifies the diffusive permeability of peritoneum for creatinine. Observed results may be clinical important.

P175 (EI0029)

In Silico Study of an Innovative Microgravity Perfusion Bioreactor for Hydrogel-Based Cardiac Regenerative Medicine

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Objectives: In cardiac regenerative medicine, hydrogel-based injectable scaffolds (hydrogel) are becoming a promising strategy for supporting the regeneration of injured heart. The rationale for this study is to assist the design of an innovative low-cost perfusion bioreactor for cell-seeded hydrogel feasibility testing, in which microgravity condition is realized by establishing a mixing slow vortex that allows adequate cell-seeded hydrogel suspension and oxygen transport without using rotating components. Computational fluid dynamics was applied to assist the bioreactor design and to identify the operating conditions that optimize mass transport in the culture chamber. **Methods:** The finite volume method was applied to simulate 3D multiphase (culture medium, cells, oxygen) fluid-dynamics, integrating calculations of diffusion, convection and consumption for assessing *(1)* the optimal geometric design, *(2)* the proper flow regime to be established within the culture chamber, and *(3)* the oxygen distribution and its consumption by cells. **Results:** Remarkable differences in the cell-seeded hydrogel distribution and suspension, in the shear stress distributions, and in the oxygen distribution and consumption arise due to variations in perfusion parameters. Our main findings are the optimization of the geometry of the chamber and the identification of a range of flow rate values that *(1)* allow cell-seeded hydrogel suspensions, avoiding sedimentation at the bottom of the chamber, *(2)* guarantee a safe range of shear stress values on cells, and *(3)* permit appropriate oxygenation. **Conclusions:** The present study allowed to properly design an innovative low-cost perfusion bioreactor (without rotating components) for cell-seeded hydrogel culture in microgravity conditions, ensuring homogenous distribution of cell-seeded hydrogel and adequate oxygen cellular consumption, and avoiding shear-induced cell damage. Findings from computational simulations will serve as criteria to set the operating conditions for future in vitro tests. The present work is carried out in the scope of BIOSCENT Project (ID 214539).

P176 (EI0018)

Hepatic Cell Encapsulation as a 3D Culture Model for the Study of Hepatitis C Virus

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Objectives: Since the discovery of hepatitis C virus (HCV), the lack of relevant cell culture system has hampered research progress on this widespread human pathogen. New approaches in tissue engineering could be a physiologically relevant hepatocyte culture model and enable a broad range of basic and applied studies to be achieved. To prolong and strengthen this breakthrough, the use of the fluidized bed bioreactor for hepatic cell cultured in alginate beads is investigated to, on the one hand mimic *in vivo* cellular conditions by the 3D environment and on the other hand, promote HCV permissiveness and viral production. **Methods:** Hepatic cells were encapsulated in four compositions (low viscosity, medium viscosity, at 1 or 2%) of alginate beads (500 µm). The growth kinetics of a human hepatocyte line, Huh-7.5.1 cells, in alginate beads were followed in dynamic condition by DNA quantification. Albumin concentration was determined by an ELISA assay to study the cell functionality. The important factors for HCV infection (HCV receptors, tight-junction, and polarity markers) were studied using immunofluorescence analysis and confocal microscopy. 3D culture of Huh-7.5.1 was infected with JFH-1 HCV and HCV-RNA levels in culture supernatants were quantified by Q-PCR. **Results:** The alginate encapsulation increases Huh-7.5.1 cell density, supports proliferation, liver function as compared to the 2D cell culture system. We demonstrate that depending of the composition of alginate, Huh-7.5.1 cells cultivated in beads formed 3D-complex, particularly aggregates and multicellular channel-like structures. This cellular organization may influence expression and relocalization of tight junction, polarity markers, HCV receptors, in comparison with 2D culture. Importantly, the multicellular structures may remain highly permissive for HCV infection. **Conclusions:** These data are encouraging to open the HCV potential accessibility to hepatocytes *in vitro*. Encapsulated Huh-7.5.1 cells may represent a promising physiologically relevant system for further *in vitro* studies of HCV life cycle, host–virus interactions.

P177 (EI0302)

Biodegradable Thermoplastic Polyurethanes: Synthesis and Characterization of Improved Tpus

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Objectives: Thermoplastic polyurethanes (TPUs) are mechanically elastomeric, have good biocompatibility, and are amenable to be processed from melt or solution. These characteristics make TPUs particularly interesting in soft tissue engineering. While biodegradation of surgical implants has been traditionally viewed as a negative characteristic, it can be a potentially very useful property if the rate of degradation is well controlled to correlate with the regrowth of native tissue. To better understand and hopefully exploit this synergetic phenomenon, our group wishes to produce biodegradable thermoplastic polyurethanes with improved mechanical properties and study the biodegradation behavior. **Methods:** To increase the tensile stength of the existing TPUs, the aliphatic diisocyanate has been replaced with a more rigid alicyclic diisocyanate. Alicyclic TPUs are expected to have comparable mechanical performance to commercially available aromatic TPUs with reduced toxicity of amine degradation products. We also tried to impact the decomposition time by varying the length of the softblock and by using a degradable polyester (caprolactone) instead of polyTHF. **Results:** The new produced TPUs are organic soluble allowing molecular weight (from 30 k to 70 k M_n) analysis by GPC. Bulk mechanical properties (modulus, tensile strength, and percent elongation) are tested in tensile tests. Simulated biodegradation is tested in-vitro with and without enzymes. A terephthalic based chain extender has already been shown to undergo slow degradation at elevated pH with prolonged degradation times of one to two years. **Conclusions:** There are many possible application areas for these new synthesized biodegradable TPUs like all areas of soft tissue engineering, drug delivery systems, disposable medical tubing, packaging and textiles.

P178 (EI0261)

Pulsatile Fluid Flow Enhances Engineered Bone Development by Human Adipose Derived Stem Cells

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Objectives: Within native bone, bone cells experience mechanical shear force due to the flow of interstitial fluid generated by physical movement. Bone tissue engineering could greatly benefit from mimicking such physiologic environment, in which the interstitial flow is dynamic and associated with fluctuating shear stress. To this end, we investigated the effects of pulsatile fluid flow (PFF) regime on bone formation in vitro by human adipose stem cells (hASCs) on porous three-dimensional scaffolds, in contrast to conventional continuous fluid flow (CFF). We also determined the timing of application of PFF that results in most rapid osteogenesis of hASCs. **Methods:** Porous silk-fibroin scaffolds (400–600 μm pore size) were seeded with hASCs (30×10^6 cells/mL) and cultured in osteogenic medium under four distinct fluid flow regimes: 1) PFF for 5 weeks; 2) CFF 1 week, PFF 4 weeks; 3) CFF 2 weeks, PFF 3 weeks; 4) CFF for 5 weeks. PFF regime was applied at flow velocities ranging from 400 μm/s–1200 μm/s, at 0.5 Hz frequency, for 2 h every 10 h, with the CFF regime during the remaining culture times. In all groups, CFF was applied at 400 μm/s. **Results:** Constructs cultured in the CFF regime demonstrated inferior bone development in terms of cell proliferation, bone protein deposition and calcification, as compared to constructs subjected to PFF. The best tissue development was achieved in group 3, when hASC were pre-differentiated for 2 weeks under CFF, and then subjected to the PFF regime, as evidenced by maximum values of bone protein deposition, calcium content, bone volume, and expression of PGE2 mechanotransduction marker. **Conclusions:** Over 5 weeks of culture, PFF improved bone formation over CFF. The response of hASCs to PFF was enhanced following pre-differentiated into the osteogenic lineage. We thus propose that hASCs develop a mechanism to detect and respond to change in shear force by progression of osteogenic differentiation.

P179 (EI0150)

A Numerical Model of Mass and Momentum Transport in Convection-Enhanced HFMBs for Long Bone Tissue Engineering

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Objectives: The synergistic effects of convection and a distributed and delocalized nutrient transport in hollow fibre membrane bioreactors(HFMBs) has been recently reported to benefit to the culture of cm-scale BMSC aggregates, possibly by relieving nutrients limitations typical of other bioreactors for bone tissue engineering(BTE). Mathematical modeling of mass transport, cells growth and metabolic reactions is particularly interesting given the difficulty to monitor non-invasively solutes concentration in the presence of a closed shell, to optimize bioreactor design and operation. Most proposed models consider cells uniformly distributed in the extracapillary space, in contrast with experimental results under high convective flows, and are then inadequate for this purpose. This paper presents mathematical models of nutrients profiles inside HFMBs operated in close shell mode from diffusion-limited to convection-dominant mass transport conditions for both uniform cell distribution and the actual non-uniform cell distribution observed in experiments with BMSCs. **Methods:** Models are based on a multi-compartment description of HFMBs based on the Krogh cylinder assumption, and on a quasi-steady state analysis of nutrients evolution and cell concentration profiles. Relevant non-dimensional parameters were identified, and governing momentum and mass transport equations were numerically solved with a finite element commercial code. Metabolic parameters for primary and immortalized cell were used, proliferation and distribution parameters were assessed from culture experiments. **Results:** The use of low metabolic requirement cell types, like immortalized ones makes the design of bioreactors for 3D constructs culture easier, being diffusion-limited nutrients transport models inadequate only for cell density close to natural bone tissue. Simulation results demonstrate the importance of convective nutrient transport, membrane permeability and packing density in the cell compartment on nutrients concentration in the ECS when primary cells are used. **Conclusions:** Convection-dominant nutrients transport is necessary to overcome nutrient transport limitation when culturing cells types with physiological metabolic requirements in 3D cm-scale constructs.

P180 (EI0211)

Preliminary Study on Development of the Series of the Hollow Fiber Membrane Bioreactors (Devoted to Different Cell Culture Applications)

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Objectives: Hollow fiber bioreactors have a broad spectrum of applications including biotoxicity testing and tissue engineering studies. Structure of the membrane should enable exchange of the biochemical compounds with required molecular mass between inner and outer bioreactor's compartments. In our institute an innovative method of modification of the standard polysulfone membranes was elaborated aiming at a controllable increase of the membranes' cut off (MWCO). The objective of this preliminary study was to evaluate filtration properties of series of the prototype, modified, polysulfone membranes designed for application in the cell bioreactor. **Methods:** Four groups of membranes: A, B, C (subgroups: C1, C2, C3) and D placed inside the bioreactor casings were tested under in-vitro condition. Modification of the membrane structure was performed based on change of the developed original technological procedure. Membranes were evaluated with saline using ultrafiltration studies (quantitative assessment) and in the separation circuit with human plasma (qualitative assessment). Sieving coefficients for glucose, albumin, IgG, HDL, IGM, and LDL were calculated and then MWCO was estimated. **Results:** The results for the ultrafiltration coefficient were as follows: 6, 28, 13, 18, 30 and 56 ml/(min*mm Hg*m^2), for membrane type A, B, C1, C2, C3 and D, respectively. The lowest value of MWCO = 80 kDa was obtained for bioreactor with type A membrane. In the remaining groups, the following MWCO values were obtained: B—350 kDa, C1—110 kDa, C2—150 kDa, C3—2000 kDa and for type D membrane MWCO was greater than 2700 kDa. **Conclusions:** The results demonstrated that MWCO increased as a response to the modification of the membrane structure (MWCO ≥ 80 kDa) in comparison to standard, not modified polysulfone membrane characterized by the MWCO < 69 kDa. It seems that developed method makes it possible to prepare the membranes with required permeability appropriate for specific bioreactor applications.

P181 (EI0411)

Platelet Lysates as a Scaffold Complement Promoting hASCs Proliferation and Osteogenic Differentiation

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Objectives: This work aims to establish platelet lysates (PL) as optimal source[1] of growth factors and other molecules that are vital for promoting cell proliferation and differentiation pathways, eventually allowing the substitution FBS and/or osteogenic supplements in culture media in bone tissue engineering strategies. Furthermore we intent to design new approaches to incorporate PLs in a scaffold material, as a hydrogel encapsulating the cells or as a coating for 3D porous structures, thus developing a tissue engineered construct with enhanced/multiple functionalities. **Methods:** Starch–polycaprolactone (SPCL) meshes were obtained by a fiber bonding method as previously described[2]. PL

gels were obtained by activation of platelets coagulation cascade using thrombin dissolved in a calcium chloride solution. Human adipose stem cells (hASCs) were obtained by enzymatic digestion of lipoaspirates samples. hASCs were either seeded directly into the SPCL scaffolds (control group) or into the scaffolds previously coated with PL gels or suspended in the PL and then seeded in the scaffold and gellified. hASCs proliferation and differentiation was assessed after different culturing time points of the constructs, by DNA and ALP quantification and by RT-PCR and immunohystological analysis. **Results:** The preliminary results obtained sustain the hypothesis that growth factors and other signaling molecules present in PL groups are actually active and vital to initiate proliferation and osteogenic differentiation of hASCs. DNA quantification and cell viability were similar and even higher in PL groups, as well as early markers of osteogenic differentiation, such as ALP activity. Latest time-points revealed less noteworthy differences especially due to the progressive degradation of the PL gel. **Conclusions:** PL represents a substrate and a delivery system of important growth factors and other signaling molecules, and therefore making these molecules available for cells within a tissue engineering construct provides an important enhancement of autologous bone tissue engineering strategies.

P182 (EI0370)

Building the Basis for Human Meniscus Regeneration

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Objectives: Total or partial meniscectomy have been the gold standard for the treatment of degenerated/diseased menisci. Despite meniscal regeneration represents a recent trend in tissue engineering, fundamental studies related to human meniscus biochemistry and biomechanics are still scarce. This work aims to contribute in the knowledge of this tissue aiming future clinical applications namely, the aspects dealing with the cellular phenotypes and density, biomechanics and extracellular matrix composition. **Methods:** Human tissue was obtained from local hospitals by means of surgery or biopsy, in accordance with local ethical committee guidelines. The HMC's were isolated from different donor (sex and age) explants or using an enzymatic standard protocol. Micro-computed tomography (Micro-CT) of freeze-dried meniscus was carried out. Histological (haematoxylin and eosin—H&E, trichrome stain and toluidine blue stainings) analysis was performed for segmental characterization of ECM and cells density. Dynamic mechanical analysis was carried out for medial, anterior and posterior segments of meniscus (in PBS at pH 7.4). **Results and Discussion:** Micro-CT analysis revealed that meniscus (freeze-dried) possessed a mean porosity of 53%, a mean pore size and trabeculae thickness of 85 μm and 80 μm, respectively. The cells isolated from meniscus are a mixed population of cells, *i.e.* fibrochondrocyte-like and MSCs. The histological evaluation has shown that meniscus ECM is composed of collagen type I. This tissue is fibrocartilagineous in nature and presented a higher cell density in the periphery as compared to meniscus core. Cellular density among the different segments (anterior, medial, posterior) of meniscus was quantified using the H&E 2-D histological images. **Conclusions:** This study has contributed to improve the knowledge on meniscus biology and mechanical properties. It is believed that these important issues should be considered to develop adequate acellular and cellular strategies for tissue engineer meniscus.

P183 (EI0079)

Combining Optics and Ultrasound to Image 3D Tissue Constructs

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Objectives: Tissue scaffolds are an integral part of the tissue engineering process, assisting in the culturing of cells in three dimensions. It is important to understand both the properties of the scaffold and the growth of cells within the scaffold. This paper describes a system to characterise scaffolds using acoustic techniques alone and the development of an ultrasound modulated optical tomography system to study the growth of cells within the scaffolds. The ultrasound modulated system allows the effects of light scattering in relatively thick tissue constructs (several mm) to be reduced. **Methods:** Acoustic techniques alone have been applied to characterise foamed scaffolds manufactured from synthetic polyesters poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) via a supercritical fluid process. An ultrasound modulated optical tomography system has been used to image absorbing and fluorescent objects in gel scaffolds. **Results:** Although foamed scaffolds are porous and therefore highly scattering to sound waves, results demonstrate that acoustic signals are detectable through a 6 mm thick foamed scaffold. Images of optically-absorbing materials embedded in gel-based tissue phantoms will be presented demonstrating that a lateral resolution of 250 μm and an axial resolution of ~90 μm can be achieved in scattering samples. Preliminary results of non-linear acousto-optic modulation will also be presented. **Conclusions:** Combining optics and ultrasound can be used to obtain high resolution optical images of highly scattering, thick tissue constructs.

P184 (EI0086)

Regulation and Characterisation of Corneal Stromal Cell Contraction

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Objectives: Collagen hydrogels have been extensively used as scaffolds for corneal tissue engineering. However, corneal stromal cells differentiate into contractile fibroblasts in the hydrogel *in vitro* culture, rather than keratocytes. The aim of this study is to develop techniques to regulate the contraction by either chemical or topographical cues which mimic the native corneal environment, and characterize the cellular feedback in prolonged culture periods via novel, non-destructive monitoring protocols. **Methods:** 5×10^5 human corneal stromal cells were seeded in collagen hydrogels with and without the incorporation of poly-lactic acid aligned nanofibers. A non-destructive spherical indentation technique was used to examine the alteration of the mechanical properties of the individual collagen hydrogel specimens under different media respectively up to 28 days. The dimensional change of the specimens caused by the cells' contraction was measured by optical coherence tomography in parallel. The quantitative-PCR with respect to the expression of keratocytic and fibroblastic markers was conducted to cross-validate the observed physical properties. It was revealed that stromal cells cultured under media with insulin and without serum exhibited constant elastic modulus and gel dimension, indicating that contraction was suppressed, which was cross-validated by the expression of keratocan and ALDH3; whilst stromal cells cultured with serum demonstrated continuously increased modulus and reduction of thickness, typical of contraction process. The presence of aligned nanofibers reduced the degree to which the cells were able to contract the hydrogel constructs in a vertical direction, thus encouraging the cells cultured in fibroblastic media to behave more like non-contractile keratocytes. **Conclusions:** The alteration of culture conditions and the addition of topographical cues can regulate corneal stromal cell differentiation. This can potentially enhance the field of corneal tissue engineering using collagen hydrogel models. The non-destructive monitoring protocols provide convenient tools for observing biological phenomenon for prolonged culture periods in the same specimen.

Tissue Engineering of Skin

P185 (EI0366)

Attachment and Spreading of Fibroblasts on Self-Assembling Bioactive Matrices

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Objectives: The primary objective of this work was to investigate the potential of 2D biodegradable membranes as supportive bioactive matrix for wound healing by studying the behavior of human fibroblasts on these membranes. Towards this goal, we developed a biomimetic matrix that incorporates structural components of skin extracellular matrix (hyaluronan) and biochemical signals (RGDS epitope) to recreate some aspects of skin tissue niche. The RGDS sequence is present in cell binding domains of extracellular proteins (such as fibronectin) and is known to promote integrin-mediated cell adhesion. **Methods:** The proposed bioactive matrices result from the self-assembly between peptide amphiphiles and hyaluronic acid (HA), a major component of skin ECM. The RGDS sequence was incorporated in the peptide structure to provide the matrices with cell-adhesive properties. Cell culture was then performed and the effect of the RGDS epitope on the adhesion, morphology and proliferation of primary human dermal fibroblasts was followed respectively by, scanning electron microscopy, immunostaining and DNA quantification. **Results:** Cell responses to RGDS matrices were compared to matrices containing DGSR (scrambled sequence that does not promote cell adhesion). When cultured on membranes without the cell recognition epitope RGDS, human dermal fibroblasts showed lower adhesion to the matrices when compared to the ones containing RGDS. SEM analysis showed adherent cells on the RGDS matrices and the presence of filopodia which are known to be involved in the regulation of cell migration. **Conclusions:** We expect that the

proposed biodegradable bioactive matrices could offer significant potential in skin regeneration strategies and also as model systems for fundamental mechanistic studies in wound remodeling. Daniela S. Ferreira acknowledges the financial support received from Fundação para a Ciência e a Tecnologia (PhD scholarship SFRH/BD/44977/2008.

P186 (EI0307)

An Arginine Incorporated Nanocomposite Polymer for Cardiovascular Implants

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Objectives: Cardiovascular implants must resist thrombosis. Polyhedral- oligomeric-silsesquioxane-poly(carbonate-urea)urethane (POSS-PCU) nanocomposite polymer has demonstrated suitable properties for cardiovascular applications. L-arginine is recognised as a significant amino acid with anticoagulant properties with a link to nitric oxide synthesis. Water soluble arginine is immobilized within the polymer via nanoparticles thus presenting a novel surface modification method for blood contacting polymers. The study aims to determine the antithrombogenic properties of Arginine-POSS-PCU. **Methods:** Arginine was reacted with amine functionalized fumed silica nanoparticles using fmoc chemistry and incorporated into POSS-PCU at 5–8% W/W. Surface properties of Arginine-POSS-PCU samples were determined using FTIR and XPS. A thorough investigation of whole blood kinetics on Arginine-POSS-PCU was performed with Thromboelastography polymer coated cups. Platelets were introduced onto Arginine-POSS-PCU samples and incubated for 90 mins at 37C on a shaker before platelet adhesion morphology with SEM and the changes in platelet activated factors were determined with ELISA. Plasma from whole blood was also introduced onto Arginine-POSS-PCU and the changes in protein adsorption were also determined using fibrinopeptideA and PreKallikein assay. **Results:** Surface presence of arginine on Argnine-POSS-PCU was confirmed. Thromboelastography tests relvealed that arginine-POSS-PCU had a lower rate of initial clot formation, (determined with K/min, R/min,α/deg) as well as longer time and a slower rate for maximum thrombus generation (TMRLG/min, MRTG/mm/min) SEM demonstrated less platelet adhesion with a more round morphology with least pseudopodia. PF4 ELISA demonstrated least activation of platelets. Fibrinopeptide A and PreKallikrein assays demonstrated that thrombogenic plasma proteins are influenced by arginine-POSS-PCU with lowered proteins. **Conclusions:** Arginine-POSS-PCU cardiovascular grafts instill greater antithrombogenic properties.

P187 (EI0305)

Liposome-Encapsulated Hemoglobin Accelerates Skin Wound Healing in Mice

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Objectives: Effects of liposome-encapsulated hemoglobin with moderately high O_2 affinity (m-LEH, $P_{50}O_2$ = 17 mm Hg) on the skin wound healing were examined in mice. **Methods:** Two full-thickness dorsal wounds 6 mm in diameter with a surrounding silicone stent were created in C57BL6J mice. Two days later (Day 2), animals randomly received intravenous m-LEH (2 mL/kg, n = 12), homologous blood transfusion (RBC, n = 11) or saline (SL, n = 12). The same treatment was repeated 4 days after wounding, while the sizes of the skin defect and ulcer were monitored on Days 0, 2, 4 and 7, when all animals were euthanized for morphological studies. **Results:** While the size of the skin defect in relation to the stent ring remained the same in all the groups, the size of the ulcer compared to the skin defect or silicone stent became significantly reduced on Days 4 and 7 in mice treated with m-LEH (46 ± 10% of pretreatment size, $P < 0.01$) compared to mice treated with RBC transfusion (73 ± 6%) or saline (76 ± 7%). M-LEH treatment significantly accelerated granulation, increased epithelial thickness, suppressed early granulocyte infiltration, and increased Ki67 expression in accordance with the ulcer size reduction, while there was no difference in surface blood flow as determined by Laser-Doppler flow meter or CD31 expression by immunohistochemical staining among the groups. **Conclusions:** The results suggest that m-LEH (2 mL/kg) may accelerate skin wound healing in C57BL6J mice via mechanism(s) involving reduced inflammation and increased metabolism, but not by improved hemodynamics or endothelial regeneration.

P188 (EI0281)

Development of Dermal Bilayered Scaffold With New Generation of Nanocomposite Polymer, with Nanosilver Outer Layer and Biodegradable Inner Layer Containing Bioactive Molecules

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Objectives: Despite the myriad of skin substitutes, current gold standard treatment of full thickness burns remains split thickness autografts. However, their use cannot be extended to patients with a large affected %TBSA (Total Body Surface Area). The objective was to develop a scaffold for dermal replacement, based on new-generation nanocomposite materials. **Methods:** A bilayered scaffold was developed; the biodegradable inner layer nanocomposite was 800 µm thick and designed with a range of porosities, the 80 µm outer, removable, non-biodegradable layer, contained nanosilver for antimicrobial protection. This scaffold underwent tensile testing, contact angle, permeability, FTIR and scanning electron microscopy (SEM) analysis. **Results:** The physical construct was easy to handle and clinically applicable. Results demonstrate the macroporosity and permeability of the scaffold, which allowed 10 ml min^{-1} of water to pass through; confirmed by SEM. Outer layer contact angle was over 90° and inner layer was <70° indicating hydrophilicity of the scaffold. Elasticity was clearly demonstrated by the Young's modulus. **Conclusions:** This promising scaffold contains many desirable properties that could successfully mimic natural skin. Future directions involve covalently bonding bioactive molecules (i.e. cyclic RGD) and seeding the scaffold with adipose tissue derived stem cells to enhance wound healing and angiogenesis.

P189 (EI0141)

Plasma Coated Electrospun Substrates for Muscle Tissue Development

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Objectives: Epicardial implantation of an engineered muscle-graft has been associated with prolonged functional recovery of the ischemic area after myocardial infarction. However, highly organised and contractile in vitro muscle tissue development strongly depends on an appropriate design of the cell culture substrate. In the present study, the effect of substrate architecture and surface functionalization on muscle cell orientation, differentiation and contractility were investigated. **Methods:** Aligned and randomly oriented micron- (3.2 ± 0.8 µm) or nano- (308 ± 178 nm) scaled fibrous polycaprolactone nonwovens were processed by electrospinning. A 15 nm thick oxygen functional hydrocarbon coating was deposited at the surface by an RF plasma process (gas mixture: CO_2:C_2H_4 ratio 6:1; power input: 50 W; process duration: 20 minutes) and characterised by XPS. C2C12 muscle cells were grown on the substrates and analysed for viability, proliferation, orientation and differentiation. Cell orientation was characterised by a cosine function, where S = 1 for parallel, S = 0 for randomly oriented cells. Myotube maturation was analysed by immunofluorescence staining of sarcomeric striation. Contractile function was assessed under electrical stimulation. **Results:** Plasma coating resulted in carboxylated, hydrophilic substrates. Cell viability varied from 40 to 60% relative to TCPS, with increased cell number on plasma coated substrates. Architectural cues highly influenced cell orientation. On aligned fibres, myoblasts displayed increased spatial orientation (S = 0.91 ± 0.03) as compared to randomly oriented fibres (S = 0.33 ± 0.2); $p < 0.01$. Plasma coating however promoted differentiation, apparent by increased myotube formation. Accordingly, most pronounced sarcomeric striation was seen on plasma coated substrates where myotubes were furthermore most susceptible for electrical stimulation and resultant contraction. **Conclusions:** The current study underlines the importance of combining architectural as well as chemical cues for advanced muscle development. Aligned fibres and plasma coating induce most pronounced myoblast differentiation. Furthermore, we provide evidence that a synthetic, fibrous substrate allows for myotube maturation and contractility.

P190 (EI0218)

Continuous Monitoring of the Feet Temperature in Patients With the Diabetic Foot Syndrome

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Objectives: Neuropathy of lower extremity is one of the complication of diabetes mellitus. The foot skin temperature is correlated with diabetic foot neuropathy. Monitoring of the foot skin temperature in diabetics may be a useful

tool for preventing foot ulceration that in serious cases may result in foot amputation. The aim of our study was to determine daily course of temperature difference between ill (with a diabetic ulcer) and healthy foot. **Methods:** Temperature measurement system developed in cooperation of the Center for Biomedical Technology (Krems, Austria) and Digilog Inc. (Perg, Austria) company was used for monitoring the skin temperature on feet. The device is able to gather 57 thousand of measurement data, which can be retrieved wirelessly using the RFID (Radio Frequency IDentification) technology. The diameter of the temperature sensor is 15 mm, and its thickness is 6 mm. The skin temperature measurements were performed every 1 or 5 minutes. Two healthy subjects and three diabetes type 2 patients were monitored for 1–6 days. **Results and Discussion:** Assessment of the temperature differences was performed in 4 periods of day: 0:00–5:59, 6:00–11:59, 12:00–17:59, 18:00–23:59. The differences were lower than 0.2 °C in healthy subjects. In diabetic patients those differences were significantly higher. The lowest temperature differences in all patients have been found during the night (0.67 °C to 1.02 °C), and the highest differences were registered in the afternoon (2.06 °C–3.36 °C). Those findings are very important in the patient's state assessment. **Conclusions:** The applied continuous temperature measurement system has been found to be feasible. Obtained results indicated significant fluctuations of temperature differences between ill and healthy foot during the day. Thus temperature as the marker of healing process has to be measured in carefully selected periods of day.

P191 (EI0190)

Reconstruction of Epidermal and Dermal Equivalent Using Human Keratinocytes and Chitosan Scaffold Containing Human Dermal Fibroblasts

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Objectives: In order to reconstruct dermal and epidermal equivalent using primary cultured normal human skin cells. **Methods:** Normal human keratinocytes (HEK) and dermal fibroblasts (HDF) were isolated from foreskin by dispase II and sequential collagenase treatment. For epidermal equivalent, HEK seeded onto porous membrane was cultured and terminally differentiated by air exposure. For dermal reconstruction, chitosan scaffold was prepared by freeze-drying of 1.5% chitosan solutions. HDF was inoculated into chitosan scaffold, and cultured for 2 weeks. **Results:** Three-dimensionally cultured epidermal equivalent was identical with normal human skin showing fully differentiated multilayer of basal layer, stratum spinosum, granular layer, and stratum corneum. Moreover, layer-specific marker expressions were also similar with human skin. Reconstructed dermal equivalent had similar physical characteristics with human dermis. It also showed porous structure of homogeneous chitosan matrix and well-spread HDF populations. **Conclusions:** Reconstructions of epidermis and dermis were achieved respectively using normal human skin cells. Further efforts will be able to reconstruct the full-thickness skin equivalent using human skin cells and chitosan scaffold.

P192 (EI0183)

Three-Dimensionally Cultured Human Nasal Epithelial Cell Sheets for Toxicity Tests and Clinical Applications

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Objectives: In order to establish the in-vitro cultured human nasal epithelial reconstruct for alternative toxicity test and even clinical applications. **Methods:** Normal human nasal epithelial cells (HNE) were isolated by dispase II treatment, and inoculated onto porous membrane. The cells were cultured under submerged until confluent, and sequentially differentiated by air exposure. Fully differentiated cell sheets were compared with intact human nasal epithelium by H&E and immunohistochemical staining. For further clinical applications, HNE were seeded onto human amniotic membrane, and its histological characteristics were also compared with human nasal epithelium. **Results:** Primary cultured HNE showed cobblestone-like morphology without contamination with other cell types. Histological observation showed well differentiated cilia. Immunohistochemical staining revealed the expression of p63 at basal cell nucleus, CD44v6 at cell-to-cell junctions of basal cell layer, Na^+/K^+ ATPase at apical layer. In addition, ColIV, LN5, CK3/12, CK13, CK5, MUC1, and MUC5AC were positively expressed. Moreover, 3-dimensionally cultured human nasal epithelial cells onto human amniotic membrane showed identical histology and marker expressions with native human nasal epithelium. **Conclusions:** In-vitro differentiated nasal epithelial cell sheets were not only suitable for alternative toxicity test due to its similarity with nasal epithelium, but also available for clinical applications.

P193 (EI0262)

Tissue Engineering of Skin: Biodegradable Scaffolds Incorporated Into Laboratory Grown Skin Substitutes

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Objectives: Extensive skin loss, such as the skin defects associated with deep burns, avulsion injuries, or giant nevi, still represents a significant clinical problem. A promising approach to treat large skin defects may be the use of a tissue engineered full thickness skin analogue with near normal anatomy and function. Apart from the crucial biological properties, such a skin substitute should exhibit adequate structural features, particularly tensile strength and optimal pliability. The goal of this study was to test whether two polymeric net-like fabrics, one already clinically established and the other one consisting of a well known clinically established material, can be used for skin tissue engineering. **Methods:** Both scaffolds were integrated into a collagen type I hydrogel and dermo-epidermal skin substitutes were generated. The skin substitutes were transplanted onto immuno-incompetent rats and analysed three weeks thereafter, employing histological analysis, immunofluorescence and scanning electron microscopy. **Results:** We found that these substitutes exhibited a well stratified epidermis that had homogeneously developed over the entire surface of the grafts. This epidermis had deposited a functional basement membrane, displayed a basal cell layer (stratum basale), several suprabasal layers and a stratum corneum. Additionally, the new skin was well vascularised even around the remnants of the supporting net-like polymer. **Conclusions:** We consider these novel dermo-epidermal skin substitutes as very promising skin analogues for the treatment of full thickness skin defect.

P194 (EI0066)

Comparative Analysis Between Acellularized and Immunologically Non-Treated Vascular Xenografts in Long-Term Survival Animals

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Objectives: We implanted acellularized and immunologically non-treated porcine xenografts as an arterial graft in goats and comparatively analyzed the explanted grafts with gross observation, as well as light microscopy and immunohistochemistry, following the predetermined periods. **Methods:** For immunologically non-treated xenografts, bilateral porcine carotid arteries were harvested, and after short-term freezing at –70°C, were implanted into goats. The preparation of acellularized xenograft vessels has been performed with Nacl-SDS solution and stored at the freezer until use. The goats were randomly assigned for five periods of observation (1 week, and 1, 3, 6, and 12 months after implantation), four animals were observed at each of these times. Periodic ultrasonographic examinations were performed during observation period. Following the predetermined periods, the explanted grafts were analyzed. **Results:** Among 20 animals, two goats died prematurely, and a total of 35 grafts were evaluated. Gross observations revealed nonthrombotic patent smooth lumens. Microscopic examinations of the explanted grafts showed satisfactory cellular reconstruction up to the 12-month observation period. The proportions of CD3 positive T lymphocytes among inflammatory cells infiltrations were very low. **Conclusions:** In conclusion, these findings, as a whole, suggest that porcine vessel xenografts can be clinically acceptably implanted in the goats as a form of small-diameter vascular graft, regardless of the acellularized xenograft or immunologically non-treated xenograft.

P195 (EI0001)

New Cardiac Bioreactor for Mechanical Conditioning of Tissue-Engineered Valvular and Vascular Substitutes

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Pathologies observed after the implantation of currently available aortic valve substitutes, such as thrombosis or accelerated tissue degradation, call for the development of an improved type of substitutes. Tissue engineering can provide, using cell culture techniques, aortic valve substitutes free of these problems, with the ability to grow, repair and remodel. In order to confer proper mechanical and biological properties to the tissue-engineered substitutes, cells must be cultured in an environment recreating hemodynamic conditions, hence the need for bioreactors. The objective pursued with the development of our new cardiac bioreactor is to reproduce, with the highest level of accuracy, any blood flow and pressure conditions up to the physiological range. The design of the bioreactor is based on a two-element Windkessel model. A diaphragm pump, driven by compressed air, is used to generate a pulsed flow within the system. A compliance chamber and resistances, for which the parameters are manually set, are used to passively modulate the pressure waveform generated. Manual optimisation of the user-defined command to the system allowed reproducing physiological flow and related pressure waveforms with

very good correlation. However, better accuracy may be achieved by closing the control loop of the system. The aim of the next prototype, which is actually under development, is then to enhance control over the reproduction of the hemodynamic conditions. An automated active component is being designed, in order to replace the compliance chamber and the resistances in their role to modulate pressure by actively modifying the behaviour of the system during each beat. Controllers using artificial intelligence algorithms, such as neural networks, are implemented to automatically control the actuation parameters of the diaphragm pump and automated component.

Latest Advances in Preventive and Regenerative Medicine Technologies

P196 (EI0351)

The Development of a Artificial Trachea

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Objectives: Our team at UCL have recently performed the first stem cell organ transplant in the world of a fully tissue engineered airway with a hugely successful outcome. For this case, a human donor trachea was harvested, decellularised and reseeded with the patient's own epithelial and mesenchymal stem cells (MSCs). The aim of this current study is to continue this research but with the use of an artificial tracheal replacement instead of delcellularised human tissue. This artificial trachea is composed of a POSS PCU polymer which has been developed in our laboratory and has undergone extensive testing, proving its huge potential for use in surgical implants. **Methods:** The biocompatibility, non-toxicity, low inflammatory response and inert nature of the polymer were established by implanting the polymer in a sheep model for 36 months. The cytocompatibility of the polymer was shown by seeding MSCs and epithelial cells onto the polymer and monitoring their attachment and proliferation using scanning electron microscopy, immunostaining and histological analysis. The structural design of the construct and specific fabrication of the polymer was determined using numerical modeling. **Results and Discussion:** The construct has proven to be biocompatible, non-toxic, have a low inflammatory response and support the attachment and proliferation of tracheal specific cell types. In addition the construct avoids luminal collapse and retains sutures. **Conclusions:** A tracheal replacement has been developed which mimics, the structure and function of the native trachea. This artificial trachea could be the first step in the use of 'off the shelf' technology leading to the potential elimination of the current organ donor shortage problem.

P197 (EI0320)

Examining Endothelial Progenitor Cells (EPCS) for Tissue Engineering Purposes with a New Shear Stress Device

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Objectives: Recent findings indicate that Endothelial Progenitor Cells (EPCs) can accelerate re-endothelialization of vessels. An intact endothelium is essential for hemocompatibility and prevention of thrombi formation. Thus, these cells are about to become an important tool for tissue engineering of grafts that have contact with blood. In our new bioreactor system we exposed late outgrowth EPCs from human peripheral blood to defined shear stress conditions and examined their behavior. **Methods:** We developed a novel bioreactor system that provides defined levels of shear stress. Basically the device consists of two coaxial cylinders with different sizes. The inner one rotates and impels medium in the gap between both cylinders which results in shear stress for the cells on the inside of the outer cylinder. Ports permit sterile withdrawal and injection of fluids during the run. Medium temperature is checked by incorporated sensors and the attached microscope system allows monitoring of cells. Expression of endothelial specific proteins PECAM-1 and VEGF-R2 was evaluated by immunocytochemistry and quantitative real-time PCR. **Results:** Using our device we were able to show differences in the expression of endothelial cell proteins in EPCs depending on the applied shear stress level. Time lapse recording of the cells not only depicted how EPCs detached during flow experiments but also revealed interesting changes in their morphology. Alteration of the cells was also prominent at mRNA transcription and protein expression levels. **Conclusions:** Our bioreactor system is a feasible device for the investigation of adherent cells under defined shear stress conditions. Interestingly, the forces of the fluid flow had influence on EPCs which was not only visible in morphological changes of cells but also at transcription and protein expression levels. Therefore more tests with EPCs will be necessary to finally figure out their suitability for tissue engineering purposes.

P198 (EI0285)

Direct in Vivo Observation of Leaflet Tissue Formation for Biovalve

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Objectives: We have developed the autologous tissue heart valved conduit "Biovalves" grown in the recipients' subcutaneous spaces, which were autonomically formed precisely according to the shape of the material molds by encapsulation with connective tissues. In this study, the formation process of the Biovalve leaflet tissue was directly observed by wireless video capsule endoscope. **Methods and Results:** The mold for Biovalve preparation consisted of five main plastic parts. The wireless video capsule endoscope was completely impregnated in one plastic part for the conduit (diameter: 16 mm). The assembled molds were placed into the dorsal subcutaneous pouches of goats. The endoscope could clearly observe the small aperture (0.5–1.5 mm) prepared between the conduit part and leaflet part with protrusion resembling the sinus of Valsalva for leaflet formation. The observation was performed every day during tissue formation for about 1 month. The air collected at the aperture at the placement was disappeared within 2 weeks. Until this time thin white solid fulfill the aperture completely. The connective tissue ingrowth started from the edge of the aperture 2 weeks after the placement. The forefront of the tissue formation accompanied with a lot of capillary. The condense tissue gradually migrated to the aperture. At about 1 month the aperture was completely covered with the tissue to form leaflet tissue. Upon removing the molds from the harvested implants Biovalve with robust collagenous leaflets were obtained. **Conclusions:** The leaflet tissue formation could be clearly observed by using wireless video capsule endoscope-embedded molds, which is one of major steps toward its clinical application.

P199 (EI0258)

The Value of Resistance Index (RI) in Progression of Diabetic Nephropathy and Chronic Renal Failure

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Objectives: Intrarenal resistive index (RI) demonstrates changes of renal vascular resistance and determines evolution in patients with diabetic nephropathy. **Methods:** Intrarenal RI values were achieved from intraparenchimal arteries; values >0.70 is considered pathologic. The study was longitudinal. Clinical parameters and renal function were evaluated at baseline and after 3, 6, 9, 12, 15, 18, 21 and 24 months. Seventy patients with diabetic nephropathy were divided based on their intrarenal RI: group 1 had values of ≥70, group 2 had values <70. A group of 30 healthy volunteers, matched for age, sex and body mass index, was used as control. **Results:** Intrarenal RI value ≥70 had 64.3%, at baseline; 50% of them had a decline in renal function after 9 months and 64% after 24 months. In patients with intrarenal RI values <70, 34% had a decline in renal function after 24 months. In multivariate regression analysis, proteinuria, higher baseline Ccr and RI were independent predictors of declining renal function. RI values were significantly affected by mean blood pressure, DeltaCCr and proteinuria. The relationship between the RI values and CCr (Delta CCr) showed a negative correlation coefficient of $r = -0.630$ ($P < 0.01$). There was no relationship between CCr and age and RI and age in diabetic patients. **Conclusions:** The RI can be used as a non-invasive, easily available parameter of the evolution in patients with advanced clinical diabetic nephropathy. An intrarenal RI value of ≥70 identifies diabetic patients at risk for progressive renal disease.

P200 (EI0221)

Hepatic Metabolism of Encapsulated Primary Human Progenitors and Hepatocytes

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Objectives: Some diseases such as drug diseases or hepatitis may affect hepatic functions and liver transplantation is often the only treatment available. To overcome the shortage in transplantable liver, implantation of primary human hepatocytes isolated from non transplantable livers can be proposed in some cases. One major limiting factor of the application is the significant cellular loss after transplantation. A new approach based on the encapsulation of primary human hepatocytes before implantation is a potential solution to enhance the engraftment of the implanted cells. It is shown in the literature that 3D culture conditions maintain the metabolic functions of mice and rat primary hepato-

cytes and enhance the differentiation of hepatic progenitors. The purpose of this project is to study the differentiation ability and the metabolic maintenance of the encapsulated primary human progenitors and hepatocytes under 3D culture in porous beads. **Methods:** Primary human progenitors and hepatocytes were obtained from surgical liver resections. The isolated cells were encapsulated in alginate and collagen beads combined or not with Poly-L-Lysine. The beads were produced using a co-axial air flow extruder (homemade design). The cells, encapsulated or not, were cultured in adapted media and their metabolism was studied at different time points. **Results and Discussion:** The metabolic functions and differentiation ability of the encapsulated cells were compared to the results obtained in 2D culture of collagen coating. Moreover, to improve the biocompatibility of the beads, a layer of Poly-L-Lysine was added on the surface of the beads. We also studied the effect of this layer on the encapsulated cell functions. **Conclusions:** Several configurations will be implanted in a rodent model, in order to reinforce the feasibility of the approach. Specific experiments will be developed to localize the position of the cells hosting beads.

P201 (EI0201)

Measles Virus Production Process for the Use in Cancer Therapy

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Objective: Measles virus which is adapted to tissue culture showed selective tumor cell killing abilities with attenuated pathogenicity. According to the dose needed for measles vaccination in cancer therapy a tenfold higher amount of the virus is needed. In this work, the production of large quantities of measles virus particles in a standardized manufacturing process should be established. First results of the work are presented here. **Methods:** Measles virus production was carried out in spinner system at 37 °C, 40 rpm and 5% CO_2. The offline tracking of cell growth was carried out by fluorescence-based and activity-based assays. The measles virus concentration was estimated by the $TCID_{50}$ method. **Results:** The cells have been adapted to a commercially available serum-free medium. In addition, all products of animal origin have been replaced. The growth surface is provided by micro carriers for the cultivation of adherent cells in stirred systems. Appropriate micro carriers were selected by comparing the adhesion capacity of the cells, the growth and the glucose consumption rates. Virus production rates showed similar results for cell associated virus and from the supernatant. In addition research on the temperature stability of the virus was carried out. It has been shown that the virus in the supernatant under culture conditions was very unstable. **Conclusion:** It is a common believe that most of the virus amount remains cell associated. This data showed new results by getting much larger amount of virus by harvesting the virus continuously from the supernatant. To meet commercial and regulatory requirements, this process must be high yielding, scalable and reproducible. These requirements are met by establishing a cell culture process employing stirred system and serum-free cell culture medium. The first results are promising for a successful scale up in the bioreactor.

P202 (EI0078)

Ester of Hyaluronic and Butyric Acid Protect Renal Cells in an in Vitro Model of Ischemic Injury

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Objectives: Acute Kidney Injury (AKI) is a rapid decline in renal function characterized by acute tubular necrosis, but also by mesangial cells (MC) proliferation and matrix deposition that represent elements of graveness and progression toward fibrosis. Recently we demonstrated in a rat model of AKI a higher improvement of renal function using mesenchymal stem cells pretreated with esters of Hyaluronic and Butiric acids (HB), compared to untreated cells. Here we investigate if HB can prevent MC proliferation, reduce matrix genes expression and prevent cell death in an in vitro model of oxidative stress, one of the main causes of AKI. **Methods:** Rat MC were pretreated with HB (1 g/l) or grown in culture media for 24 h, before adding H_2O_2 50 µM for 6-16-24-48 h to induce an oxidative damage. MC proliferation was assessed by cell cycle analysis; MMP-9 and collagen-1 gene expression was evaluated by Real-Time PCR. Apoptosis and necrosis were assessed by analysis of caspase-3 activity and lactate dehydrogenase release. Investigation of involved pathways (Akt and p38) was performed by Western Blot. **Results:** At 24 h, the 35% of H_2O_2-group is in G2/S phase, compared to 10% of control and 23% of HB-group. The reduction of proliferation is joined by a significant decrease in Akt phosphorylation. HB induces a significant increase of MMP-9, involved in matrix degradation, at every time considered, and a reduction of Collagen-1 expression at 24 h, compared to H_2O_2-group. H_2O_2 treatment causes necrosis, that is strongly inhibited by HB. At 16 h caspase-3 activity is higher in HB group, indicating that in this case cells die for apoptosis, as confirmed by P38 phosphorylation, preventing the inflammation necrosis-related. **Conclusions:** We demonstrated that HB protect MC from injuries induced by oxidative stress, suggesting its employment as first aid to rescue a damaged kidney, that may be followed by delayed transplantation of stem cells.

New Biomaterials and Scaffolds

P203 (EI0386)

In Vitro Magnesium Degradation with Variation of Flow Rate and Pressure

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The use of magnesium as a bioresorbable material for clinical applications is very interesting because of his mechanical and biocompatibility properties. Magnesium is not in use in medical applications, because the degradation mechanism and the mechanism of some observed effects are not yet fully understood. A review revealed that the comparison of literature data is not possible, because many studies deal with different methods like static, electrochemical and some dynamic techniques with a wide range of different test fluids and test parameters. **Objectives:** The effects of the magnesium degradation and its degradation in a biological environment are not yet understood. The focus of this project is to investigate the degradation mechanism of these effects and to improve the degradation behaviour of magnesium for implantation purposes. **Methods and Results:** With the objective of the standardization of degradation studies a dynamic in vitro test system was developed. Temperature, flow rate, and pressure of the fluid can be controlled in this system in order to investigate their influence on the degradation process. Within ongoing studies, the dynamic degradation system depicts the influence of flow rate and static pressure on the degradation process in distilled water and saline solution. The degradation process is analysed by measuring the mass loss, the hydrogen release and the Mg^{2+} concentration in the solution. **Conclusion:** Since the magnesium degradation process and the interaction between the biological environment is not exactly understood, the first step towards standardization of degradation studies has been done by classifying the influence of the flow rate and the pressure on the degradation rate. In further studies various model fluids, which are described in the literature, will be tested in this new set-up. This project is funded by the German Research Foundation within SFB 599 "*Sustainable bioresorbable and permanent implants of metallic and ceramic material.*"

P204 (EI0332)

Using Lacunarity to Characterize Pore Distribution in Scaffolds

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Objectives: The performance of scaffolds for cells growth is largely influenced by their physical properties. In particular, it is well known that porosity plays a major role. However, the characterization of the spatial distribution of pores in irregular scaffolds is a challenging task, when the pores distribution is not homogeneous, with pores either clustered or dispersed. Here we propose lacunarity (LAC) as a metric suitable for the characterization of the spatial distribution of pores in scaffolds for tissue engineering. **Methods:** Submicron resolution microCT images of a commercial scaffold (Chondro-Gide, CG) were used. From them, synthetic images with the same porosity as the CG, but with different spatial distributions of pores, were generated. On both recorded and synthetic images LAC was calculated, in order to measure the spatial distribution of pores. Lacunarity analysis is a multi-scaled method of determining the texture associated with patterns of spatial dispersion. Lacunarity provides an analysis of scaffold images in terms of (1) the overall fraction covered by the attribute of interest, (2) the presence and scale of randomness and (3) the existence of hierarchical structure. **Results:** WE observed that in scaffolds with the same macroscopic porosity value the greater the degree of pore clusterization, the higher the value of LAC. In fact, in synthetic scaffold images with porosity equal to 50% and with high clusterization, LAC is 1.83, while in the presence of dispersed pores LAC is reduced up to about the 25%. Interestingly, we conjecture that the CG is characterized by dispersed pores, because we calculated a LAC equal to 1.38 on its microCT images. **Conclusions:** Our findings clearly show that LAC can be a powerful tool for scaffolds properties characterization. In the future, pore clusterization will be analyzed to get insight into its influence on physical parameters such as permeability.

P205 (EI0212)

Blood Permeability Examination of Different Sealing Materials for Polyester Vascular Prosthesis

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Objectives: The aim of this study was determination and comparison of blood permeability of different sealing materials for vascular grafts. **Methods:** The material of the investigation were sealings based on microcrystalline chitosan, PHB or albumin-dextran mixtures applied on Bard 004187 vascular grafts. As an operating medium antycoagulated CPDA-1 porcine blood was utilized. Innovative measurement methods of blood permeability through vascular grafts in relation to the standard ISO 7198:1998(E) were designed. A static test was performed for all sealing materials. The dynamic test was fulfilled for sealings with the lowest permeability. The static test consisted on generating hydrostatic pressure of 100 mm Hg in the sample by putting the blood reservoir 1.2 m above the sample. The dynamic test consisted on placing the graft samples in a mock circulatory system were pulstile pressure of 110 ± 60 mm Hg was generated. The time and the amount of collected blood were determined. **Results:** For each sealing type different compositions of the sealing layer were examined. For six microcrystalline chitosan sealings the blood lost varied from 0.7 to 60.7 ml/min/cm^2 and for nine PHB sealings varied from 50.69 to 613 ml/min/cm^2. Tree albumin-dextran sealings showed no blood lost. In the dynamic test the albumin-dextran sealings showed a blood lost less than 0.01 ml/min/cm^2. **Conclusions:** No sealing properties were observed in relation to PHB derivates sealings. Different sealing potential characterized microcrystalline chitosan sealings. The best sealing effect was observed for vascular grafts sealed with dextran-albumin mixtures.

P206 (EI0193)

Recent Results of Novel Silicone Hollow Sphere Fibers (SHSF) for Membrane Oxygenator Modules

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Objectives: Major limitation of conventional silicone hollow fibers (HF) once compared with micro-porous HF is poor gas permeability. However, unpredictability of plasma leakage, foam formation, and brittleness elevate risks of blood trauma, thus restricting long-term application of micro-porous fibers in ECMO. Limitation of the poor gas permeability in silicone is difficult to overcome by fabricating fine, thin hollow fibers for the reduction of resistance. We introduce a novel type of pure diffusive silicone capillaries (SHSF) with walls embedding micro spheres: By enclosure of such micro spheres, a high gas exchange performance is established due to resistance reduction, in addition to adequate stability by preserving the membrane wall thickness. **Methods:** SHSF are crosswound into cylindrical silicone flexible housing. SHSF (RAUMEDIC, AG) with 200–400 µm and 100 µm wall thickness embracing 40% ratio of embedded air spheres to impose an optimal compromise between structural stability, and gas exchange efficacy. Small-scaled oxygenator-modules for low flow rates using SHSF, as well as conventional HF as control modules were constructed for In-vitro validation in a custom-built experimental circuit. **Results:** 11 different module types, besides 2 control modules made of conventional microporous fibers were constructed**.** As an example: The smallest module has a priming volume of 1.6 ml. It contains 200 fibers, and an effective length of 125 mm. Membrane surface area amounts 0.02 m^2. Water as fluid phase is used in order to preserve fibers for subsequent reproducible testing. The inlet PO_2 value of 70 mm Hg can be oxygenated up to 430 mm Hg, leading to an elimination PCO_2 difference of 16 mm Hg at a flow rate of 50 ml/min. Preliminary results with whole blood demonstrate the modules' efficiency. **Conclusions:** Results obtained indicate that SHSF represent a promising alternative to both conventional micro-porous and silicone HF. Further experiments with blood will be the next step for more tangible validation of efficacy.

P207 (EI0082)

Composites of Hydroxyapatite Doped with Nano-Powder of Titanium Oxide

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Objectives: Hydroxyapatite (HA) is currently the most demanded biomaterial for reconstructing human skeleton. Ceramics based on HA have been successfully used for many decades in medicine. Nevertheless, the poor mechanical properties of HA prohibit HA's application in the areas of the skeleton which undergo high mechanical loading. The mechanically weak HA can be reinforced with different oxides after sintering. Usually micro-sized reinforcement materials have been used but there are few publications reporting the addition of nano-sized reinforcement materials. Since earlier studies showed that Ti containing composites are highly biocompatible in cell cultures, this study determined the structure and the mechanical properties of bovine derived hydroxyapatite (BHA) doped with nano-powder of TiO2. **Methods:** Composites of calcinated BHA and nano-powder of TiO2 were prepared by adding 5 and 10 wt% of TiO2 in BHA powder. The powders mixtures were homogenized with ball-milling, pressed into pellets, and finally sintered at different temperatures between 1000°C and 1300°C for 4-hours in air. Microstructure observations with SEM and density measurements were carried out along with compression strength and microhardness tests. **Results:** The sintered samples comprised HA and Ti-oxide associated phases. The compression strength and the microhardness of the HA-TiO_2 composites increased with increasing TiO_2 content and sintering temperature (up to 1300°C); the highest values were 139.95 MPa and 316.5 HV, respectively (for 10%TiO_2 and 1300°C). **Conclusions:** The high mechanical properties of the produced composites in conjunction with microstructure features and in the light of their earlier reported biocompatibility, qualify them further consideration in developing promising new bioceramics.

P208 (EI0098)

Production of Bioceramics Nano-Particles from Eggshells Waste

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Objectives: Eggshells approximately comprise 96wt.% mineralized phases, which predominantly consists of $CaCO_3$, and 4wt.% organic matter; minor amounts of other oxides also exist. It is very easy to collect eggshells from home, bakery shops, food factories etc. Eggshells can be regarded as a very easy and pure source for hydroxyapatite (HA) production via various hydrothermal processing methods. The aim of this study was to produce nano-particles of HA via other simple methods, specifically ultrasonic and hotplate methods. **Methods:** Eggshells were collected and cleaned by deionized water from organic mater and dried. They were subjected to ball milling until powder of 100 µm was obtained. The powder was subjected to DTA analysis to determine the content of $CaCO_3$ and thereby the equivalent amount of phosphoric acid needed to be added to form TCP and HA via ultrasonic and hotplate methods. The obtained powders were sintered at 450°C and 850°C for 4-hours in air. The sintered bodies were characterized with IR and SEM analysis. **Results:** Ultrasonic agitation stimulated the reactivity of chemical species through particle size reduction and surface activation by intensive stirring, resulting in the acceleration of the heterogeneous reactions between liquid and solid reactants effectively and taking extracts from liquid phase. Hotplate method enabled HA formation. x-ray diffractograms indicated various phases of TCP and HA formed after 4-hours sintering at 450°C and 850°C. **Conclusions:** Ultrasonic and hotplate methods feature safety and versatility with regards to fabricating HA and other similar phases. The low cost and the possibility to produce nano-powders of HA and TCP are two more very attractive advantages of these techniques.

P209 (EI0094)

Microstructure and Mechanical Properties of Sintered Sheep Enamel Derived Hydroxyapatite

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Objectives: Calcium phosphate ceramics are popular materials for bone reconstruction and reinforcement for a long time. Hydroxyapatite (HA), one of the calcium phosphate ceramics, has been successfully applied in medicine due to its excellent biocompatibility with hard tissues. Since its chemical and crystallographic properties closely resemble those of bone and tooth minerals, HA attracts a particular interest for bone grafting, augmentation in maxillofacial surgery and in orthopedics as space filling material. There is limited literature information on using bovine enamel as graft material. Moreover, there are still no attempts to use sheep enamel (and dentine) HA as a graft material. The aim of this study was to investigate the structure and the mechanical properties of bioceramics from sheep derived enamel HA. **Methods:** Bovine teeth were collected and cleaned from fatty tissues. Then they were subjected to calcination at c.a. 850°C. Enamel and dentine parts were separated easily. Enamel parts were wet ball milled until fine powder (100 µm) was produced. The dried powders were dry pressed to cylindrical green samples, suitable for compression test. The samples were sintered for 4-hours in air at several temperatures between 1000°C–1300°C. Microhardness, compression strength and density measurements along with x-ray diffraction analysis and SEM observations of the sintered samples and statistical-tests were realized. **Results:** The best values for compression strength and microhardness were obtained for the samples sintered at 1300°C, namely 100.17 MPa and 366.36 HV, respectively. These results agree fairly well with the density measurements as well as the crystallographic analysis and the microstructure observations. **Conclusions:** The comparison of the results of this study with those obtained from earlier studies where HA was derived from bovine bones (BHA) or BHA composites shows that the sheep derived enamel HA results in superb biomaterials. Moreover, this study proposes a production of HA from an economic and natural source.

P210 (EI0091)

Hydroxyapatite Production with Various Techniques From Sea Urchin

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Objectives: Natural species of sea origin, such as corals and nacres, always attract special interest in biomaterials science and technology because of excellent biological properties. Their bio-mineralization mechanism has been extensively investigated and documented. Nevertheless, there are only few studies dealing with the use of sea urchins as biomaterials. The aim of this study was to fabricate various types of biological active hydroxyapatite (HA) nanoparticles with various methods. **Methods:** Sea urchin skeletons were collected from beaches of Marmara Sea and then brushed, washed, and dried. The particles were milled until fine powder of 100 µm was produced. DTA analysis results were used to calculate the equivalent amount of phosphoric acid needed to satisfy the stoichiometry of HA. The collected powder was rinsed into distilled water. For the hydrothermal transformation, the suspension was put separately to ultrasonic bath and hotplate. After 15 minutes, the equivalent phosphoric acid was added drop by drop. The treatment was continued (either in the ultrasonic bath or in the hotplate) for 2 more hours. Then, the precipitates were removed from the suspension. After drying, the powders were sintered for 4-hours in air at 450°C and 850°C. The sintered bodies were analyzed with FTIR, x-ray diffraction, and SEM. **Results:** The sintered bodies contained various phases of HA and TCP, as revealed form the XRD and IR analysis. Fine microstructures of nano-sized grains were observed with SEM. **Conclusions:** This study presented easy production methods of HA with hotplate and ultrasonic. Thus, conventional hydrothermal methods, such as those which employ high pressure vessels, which could be very dangerous, tedious and expensive, can be omitted.

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P211(EI0074)

Microstructure and Mechanical Properties of Composites of Bovine-Derived Hydroxyapatite (BHA) Doped With Nano-Powder of Lanthanum Oxide

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Objectives: Hydroxyapatite (HA) is one of the most widely used biomaterial for skeleton reconstruction since decades. HA materials are very popular for bone restorations because they accelerate bone growth around the implant due to their chemical and crystallographic similarity to human carbonate apatite. Nevertheless, HA ceramics are not used in load carrying applications because of their poor mechanical properties. To improve the mechanical properties of HA-bioceramics, such as microhardness and compression strength, metallic materials, various ceramic oxides, or whiskers, can be added in HA for fabricating composites. The aim of this study was to investigate the structure and the mechanical properties of composites of bovine derived HA (BHA) doped with nano-powders of lanthanum oxide. **Methods:** BHA was mixed separately with 5 and 10wt% nano-powder of lanthanum oxide with dry ball milling for 4-hours. Green bodies of cold-pressed samples were sintered in air for 4 hours at different temperatures (1000–1300°C). Density, microhardness, compression strength, x-ray diffraction and SEM observations were conducted. **Results:** The best values of compression strength and microhardness were obtained for the samples sintered at 1300°C (specifically 130.75 MPa and 385.54 HV for 5% doping and 123.28 MPa and 434.45 HV for 10% doping). These results are consistent with the density measurements, the results of x-ray diffraction analysis and the SEM observations. **Conclusions:** Provided that small amounts of lanthanum can be safely incorporated in biomaterials without jeopardizing bioactivity, the addition of the nano-powder of lanthanum oxide in the matrix of biological HA seems to result in a composite structure which is resistible against loads.

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Modeling in the Cardiovascular System

P212 (EI0318)

A Variable Elastance Based Mock Circulation Model for Replicating Human Cardiovascular System

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Objectives: The objective of the paper is to develop a mock circulation loop which can represent (i) the left and right parts of the heart and (ii) systemic and pulmonary vascular branches. The loop has variable elastance based left and right ventricular actuation. The system will be configurable to model congestive heart failure for testing of left, right and bi-directional ventricular assist devices (LVAD, RVAD, Bi-VAD). **Methods:** A software based cardiovascular model is run in parallel with the mock circulation. Left and right ventricular volumes and elastances are supplied as reference form the cardiovascular model to the loop. Hydraulic pumps will use them to repeat the pulsatility of the ventricles. The other compliances will be modelled by passive closed to atmosphere (arterial) windkessels and open (atrial and venous) chambers. Vascular resistances will be modelled by pinch valves. **Results:** Two sets of results will be presented; these are the pressures, ventricular volumes and systemic arterial flows of (i) healthy human dynamics and (ii) dynamics for end stage congestive heart failure. These reflect the changes in system parameters of ventricular elastances, vascular resistances and systemic arterial compliances from healthy to ill mock circulations. **Conclusions:** A mock circulation is developed to replicate healthy and ill dynamics of a cardiovascular system. The system has more realistic presure and volume signals as a result of variable elastance based excitation from a cardiovaascular model.

P213 (EI0266)

Highly Transparent Hollow Models for Flow Visualization—A Sweet Secret

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Aims: For visualization of flow and the interaction between cardiovascular structures and prostheses, transparent models with excellent optical qualities are necessary. Such models are mostly made from casts starting with positive prototypes created by CNC-milling or 3D-printing. To get the final transparent model an intermediate dead-mould from an easily removable material is required. This material must allow casting of perfectly smooth surfaces and must not interact with the surface of the final silicone material. Usually, for this purpose either wax or low-melting metal is used. However, wax tends to penetrate the silicone and so destruct the surface. Low-melting metals are expensive or toxic. We developed a method, based on chocolate. **Methods:** To create models of the aorta, first a positive prototype of the inner aortic surface was printed on a 3D-printer (Eden350 Objet-Geometries Ltd., Israel, Material: FullCure720). This model was then molded with a silicone negative form, which was devided into two halves. After removing the positive prototype, the silicone casting mold was filled with baking chocolate. The chocolate aorta was coated with colored polyvinyl alcohol and then casted with highly transparent silicone (Sylgard184, Dow-Corning Corp. MI, USA). After curing, the chocolate was melt out by hot water. **Results:** The created models were of perfect transparent quality for PIV with 20um-particles and for videotaping valve interactions. Eventual modifications could be easily performed. With a refractory-adapted blood mimicking fluid (glycerine/water/Na-jodide) an adaption up to even invisible boundary surfaces could be achieved. **Conclusion:** The described process is a cheap and effective way to create transparent models with excellent optical quality.

P214 (EI0170)

Liposome-Encapsulated Hemoglobin Ameliorates Bronchial Anastomotic Healing after Radiation and Pneumonectomy in the Rat

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Objectives: Liposome-encapsulated hemoglobin (LEH), an artificial oxygen carrier, has been reported to accelerate surgical wound healing. In the current study, LEH was tested in bronchial anastomotic healing after radiation and pneumonectomy as an experimental model of combined treatment for lung cancer. **Methods:** SD rats received preoperative radiation (20 Gy) to the chest as a simulation of cancer radiotherapy. Four days later, all animals underwent the left pneumonectomy with the bronchial stump closure (Sweet method) using 3 stitches of 7-0 monofilament suture. At the operation, rats were randomly assigned to receive intravenous infusion of LEH with high O_2 affinity ($P_{50}O_2$ = 17 mm Hg, 10 ml/kg, n = 17) or saline as a control (10 ml/kg, n = 19). Additional rats (n = 33) were treated in the same way without preoperative radiation. Bronchial anastomotic healing was evaluated 2 days after surgery, when the animals were sacrificed to determine bursting pressure of the bronchial suture line. **Results:** In rats with no preoperative radiation, bursting pressure was equivalent 2 days after pneumonectomy regardless of LEH-treatment (154 ± 94 mm Hg, n = 17) or saline control (138 ± 81 mm Hg, n = 16). In rats pretreated with radiation, however, LEH was significantly more effective in preserving the bursting pressure (150 ± 42 mm Hg, n = 17, P = 0.044) than in radiated control rats receiving saline (110 ± 61 mm Hg, n = 12). **Conclusions:** While the effect of LEH (10 ml/kg) with high O_2 affinity ($P_{50}O_2$ = 17 mm Hg) was not significant in untreated rats, mechanical strength in bronchial anastomosis was significantly preserved by LEH-treatment in rats pretreated with radiation. The results suggest that LEH may be protective of bronchial anastomotic healing in compromised host by preoperative radiation therapy.

P215 (EI0254)

Purpose of Parallelizing Cannulas of Obese Patients on ECMO Support

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Objectives: Obese patients have a significantly higher risk of stroke complications during ECMO support. That risk is connected with advanced state of atherosclerosis. Larger patients need higher perfusion flow which needs high pressure in the ECMO set. Then the flow from outflow cannula are of high energy which could result in releasing of atherosclerotic plaques. **Methods:** Best way to lower systemic pressure is to lower the resistance of junction between patient and the ECMO system. This resistance is formed on inflow/outflow cannula. Inspired from electrical analogies, we tried to lower the pressure by parallelizing the inflow and outflow cannulas. We designed a simple model to illustrate this approach. **Results:** In ideal case the pressure is two times lower with parallel connection than with standard connection. That positively influences the blood energy in outflow cannula and lowers the risk of releasing atherosclerotic plaques. We are aware of complication associated with higher surface area and implementation of other cannulas, but we are convinced, that the benefits are for obese patients more important. **Conclusions:** Parallelizing the inflow and outflow cannulas is promising way how lower risk of stroke complication during ECMO support of obese patients.

P216 (EI0151)

Theoretical Prediction of Alveolar Gas Fractions Using Variable Artificial Dead Space Volume

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Objectives: The Dead space volume (V_D) is the volume of respiratory gas which does not cooperate in the pulmonary gas exchange; such volume tends to rise in the case of patients undergoing anesthetic conditions e.g. tracheal intubation, mechanical ventilation, and also for therapeutic reasons for improvement of lung function, thus causing changes within the alveolar gas content and in turn affecting blood-gas content. Here, we illustrate a theoretical model (based on experimental validation) enabling prediction of alveolar gas composition according to overall extended V_D. **Methods:** Numerical balancing is applied according to a 3 step method taking into consideration respiratory parameters. The first step is air entering to the alveolar level. Second step is by applying the alveolar gas transport equations and the third step is balancing at atmospheric level, equivalent to alveolar gas volume (O_2 or CO_2) mixed with dead space gas volume. The respiratory volumetric parameters are measured (Wright Respirometer) and the partial gas data as well as gas fractions are monitored (Radiometer, Draeger). **Results:** According to computational balancing by maintaining a Tidal Volume up to 750 ml and a breathing frequency of 14breaths.min^{-1}, an increase of 450 ml V_D will result an alveolar CO_2 partial pressure P_{ACO2} = 42.25 mm Hg. Such results showed accordance with arterial gas data reported in the literature by maintaining the same respiratory conditions which is an arterial CO_2 partial pressure P_{ACO2} = 42 mm Hg. **Conclusions:** It's demonstrated that the theoretical approach is in accordance to the laboratory measured data and can be used for practical applications such as optimizing of e.g. functional dead space as respiratory tubes for therapeutic but also for sport training purposes.

P217 (EI0152)

CFD Modeling of Rotary Blood Pump

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Objectives: The aim of this paper was calculate hydrodynamical stresses in rotary blood pump. **Methods:** We have used CFD method for calculations of hydronymical stresses and energy dissipation distribution in few different geometry of rotary blood pump. **Results:** As a results we have obtained color maps of pressure, velocity, stresses as well as energy dissipation. On the basis of this maps we are able to predict wchich pump will cause less hemolysis. **Conclusions:** Analyzing of obtained results we can state that the least hemolysis causes pump with canal propeler. We have noticed the least energy dissipation as well as area of large energy dissipation was the smallest for such a pump.

P218 (EI0250)

Model of the Arterial System with Integrated Myogenic, Metabolic, and Endothelial Peripheral Controls for the Comparison Between Continuous and Pulsatile Perfusion During Cardiac Surgery

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Objectives: Models used to simulate non-physiological cardiovascular conditions are usually passive models, but the effect of peripheral local regulation are not negligible when studying alterations in vascular system physiology that imply oscillations in microvessel diameter. A large literature is available about myogenic and metabolic controls, while only one model allows to separately evaluate the effects of endothelial regulation. This work aims to develop a single controlled model useful to evaluate the variations in micro-vessel diameter during cardiac surgery when continuous or pulsatile perfusions are used. **Materials and Methods:** Control mechanisms have been integrated and interfaced with a lumped-parameter model of the systemic circulation, consisting of large artery segments and peripheral networks. Myogenic and endothelial controls act on arterioles, metabolic control acts at venules level. Particular attention was paid to the integration of the simultaneous action of myogenic and endothelial control on arterioles. The model was developed using Visual C++ language and LabVIEW™7.1 software was used for the graphical interface. The effects of either single control activation or multiple controls simultaneous action were evaluated under physiological and pathological conditions. The model was applied to study the variation in peripheral vascular diameters under continuous or pulsatile perfusion in order to explain the clini-

cal evidences of poor organ perfusion under continuous or pulsatile cardiopulmonary bypass. **Results:** The developed lumped parameter controlled model allows to simulate both physiological and pathological conditions as well as cardio-surgical procedures. According to the experimental evidences, the main results obtained from the model revealed a widespread vascular constriction under continuous perfusion with respect to pulsatile. **Conclusions:** The developed model appears flexible and reliable allowing to highlight the effects of local control mechanisms that play a leading role when pathologies or cardio-surgical procedures were considered.

P219 (EI0113)

Virtual Respiratory System in Evaluation of Clinically Applicable Models

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Objectives: Models that can be directly applicable in clinics are usually simple because of difficulties in fitting a complex model to data obtained for individual patients. Therefore, the simplest model of the respiratory system (RS), the RC-model with the resistance (R) and compliance (C) represented by two single numbers, is most frequently used in both diagnosis and treatment. Since, however, the true RS is much more complex and sophisticated than simple models (e.g. nonlinear and/or changeable properties of RS), it should be tested each time whether a model is appropriate for a particular clinical application or not. As the use of real human beings in experiments is limited by serious legal and ethical restrictions, virtual patients (an aggregate of comprehensive models) can be utilized for such tests. **Methods:** The virtual cardiopulmonary system elaborated previously in the Institute of Biocybernetics has been used in some of such tests. **Results:** The system was used to determine when the RC-model could be helpful in spirometry interpretation. The most fundamental questions are: (1) which parameters of the real RS influencing spirometry can correspond to the model parameters R and C, (2) how to examine an individual patient to make this correspondence actual. Virtual examinations showed that physically measured resistance and compliance depend on many measurement conditions, and thus—depending on these conditions—the same patient may be characterized by the RC-model having various values of R and C. Certainly, such a result cannot have clinical meaning, at least in spirometry interpretation. Virtual experiments showed that, for example, measurement should be performed in such a way to connect R with resistance of airways after maximal inspiration. **Conclusion:** Virtual patients may be used instead of real patients in initial evaluation of simpler but clinically applicable models and measurement methods.

P220 (EI0032)

Multiscale Modeling of the Human Liver Blood Circulation: From the Hepatic Macrovasculature Towards the Microvasculature

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Objectives: Computational models of an organ's vasculature may provide insight into organ hemodynamics, perfusion and (dys)function (e.g. transplant research). Previously, we developed an electrical analog model of the human hepatic circulation, based on measured anatomical data of the macrocirculation. However, this model requires refinement, especially at the microcirculatory level. **Methods:** Vascular corrosion casts of two human livers (discarded for transplantation) were obtained by simultaneous injections of Batson's #17 through the hepatic artery (HA) and portal vein (PV). We previously reported data obtained from an in globo micro-CT scan (resolution ± 110 μm) of one of the liver casts. As this only allowed to assess 5–6 generations, we dissected a lobe and a small sample (±0.134 mm^3) from the cast and scanned these at higher resolutions (±71 μm and ±2.6 μm, respectively). Image processing was performed to obtain 3D reconstructions up to the terminal microcirculatory level. These reconstructions enabled measurements of the branching topology (diameters, radii, lengths), and assessment of the microvascular porosity (void volume divided by total volume). **Results:** The dissected lobe dataset resulted in the visualization of higher order blood vessel generations (13 for the HA and PV, 10 for the hepatic veins (HV)). Exponential relations ($y = a\ \exp(bN)$ with N = generation number) were determined based on data from the 1st to 13th/10th generation, relating generation number to radius, length and number of vessels. For the HA/PV/HV radii [mm], a was 4.22/10.26/13.52, while b was –0.31/–0.37/–0.48, respectively. The smallest sample showed a very complex network of interconnected and intertwined sinusoids (diameters of ±5–10 μm), and the porosity was estimated at 0.148 ± 0.007. **Conclusions:** We gathered anatomical and morphological data on the hepatic macro- and microcirculation, forming the basis of a multiscale model of the liver blood flow and perfusion. The application of this method could also be extended to other organs, such as kidneys.

Natural-Based Polymeric Biomaterials and Composites for Regenerative Medicine

P221 (EI0369)

Novel Gellan Gum Hydrogels for Tissue Engineering of Intervertebral Disc

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Objectives: Centrally situated in the intervertebral disc (IVD) structure, nucleus pulposus (NP) is a gel-like tissue containing proteoglycan (PG) such as versican, biglycan, decorin, fibromodulin, luminican and especially aggrecan. NP has an important structural role in the IVD, and upon damage it possesses a limited self-repair capacity. Therefore, the main purpose of this work was to develop novel gellan gum-based hydrogel (GG) formulations consisting of GG MPs dispersed in a GG matrix for finding application as a NP substitute. **Methods:** Several GG MP/hydrogels discs formulations were prepared by means of mixing high and low acyl GG at different ratio, namely 75%:25% (v/v); 50%:50% (v/v), 25%:75% (v/v); HAGG 0.75% and LAGG 2%, respectively. The GG MP/hydrogel discs formulations were investigated by dynamic mechanical analysis (DMA), swelling behaviour and degradation rate. The possible cytotoxicity of MP/hydrogel discs leachables was screened *in vitro* by means of using a rat lung fibroblast-like cell (L929 cells) line. In order to qualitatively investigate the encapsulation efficacy of L929 cells into the GG MP/hydrogel discs a Live/Dead cell viability assay was also carried out. **Results and Discussion:** The developed GG MPs/hydrogel discs were physico-chemically characterized by FTIR and ^{1}H-NMR, and GG MPs size was measured by a stereo microscope by means of staining the MPs with Toluidine Blue-O. From DMA analysis, we observed that the optimal outcome to reinforce GG matrices may be in the range of 50–500 mg/mL of incorporated MPs. The cell culture studies demonstrated that MP/hydrogels discs are non-cytotoxic over L929 cells. Complementarily, it was also demonstrated that L929 cells can be successfully encapsulated in the GG MP of different formulation and that were viable after 72 hours of culturing. **Conclusions:** The developed GG MPs/hydrogel discs are promising hydrogels for being used in IVD tissue engineering applications.

P222 (EI0323)

Subcritical Sintering of an Aliphatic Polyester Enriched with Ulvan Capsules Loaded With a Bioactive Agent

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Objectives: A marine derived polysaccharide, ulvan, extracted from green algae, was combined with Poly-DL-Lactic acid (PDLLA) in order to produce a novel scaffold targeted for Tissue Engineering (TE) applications. The combination of this natural origin polysaccharide with PDLLA aimed the improvement of the scaffold's performance and physical integrity while broadening drug release capabilities. **Methods:** 3D scaffolds of PDLLA loaded with ulvan beads were prepared by subcritical fluid sintering with carbon dioxide at 40°C and 50 bar. The prepared matrices were further characterized by several techniques as to assess their feasibility as scaffolds for bone regeneration, namely mechanical compression testing, water uptake and degradation studies, morphological characterization by micro-computed tomography (μ–CT) and scanning electron microscopy (SEM) and standard biocompatibility tests (ISO/EN 10993). Effectiveness of Ulvan particle loading with dexamethasone within the PDLLA matrix was monitored in terms of release profile of the compound by UV spectroscopy. **Results and Discussion:** PDLLA/ulvan 3D structures exhibited a compressive modulus of 8.3 ± 1.51 MPa, a maximum water uptake of 8% and weight loss of 5%, after 21 days. In terms of morphometric properties, these scaffolds presented a porosity of 75.7 ± 1.24 %, pore interconnectivity 48.7 ± 8.49 % and a mean pore diameter of 268.5 ± 12.02 μm. The release of dexamethasone from the ulvan particles demonstrate that the systems designed can be successfully used for in situ delivery of bioactive agents. **Conclusion:** The obtained results demonstrated the feasibility of processing PDLLA/ulvan 3D scaffolds for potential TE scaffolds. These structures presented appropriate mechanical and morphological characteristics to be used as a scaffold for cancellous bone tissue engineering. They also contributed to the establishment of ulvan as an emerging biomaterial candidate for TE applications.

P223 (EI0394)

In Vivo Study on the Angiogenic Potential of Gellan Gum-Based Hydrogels for Application in Nucleus Pulposus Regeneration

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Objectives: Tissue engineering of nucleus pulposus (NP) offers a promising alternative strategy to current ineffective clinical approaches for treating intervertebral disc degeneration. Gellan gum-based hydrogels (ionic- and photo-crosslinked methacrylated gellan gum) have been recently proposed as potential candidates for NP regeneration. An important feature of these hydrogels will be their capacity to control blood vessel growth, since the NP is naturally avascular. Our aim was to investigate *in vivo* the angiogenic/antiangiogenic potential of the developed hydrogels, using an optimized adaptation of the chorioallantoic membrane (CAM) assay. **Methods:** Sterile hydrogel discs (n = 10) made of gellan gum, ionic- and photo-crosslinked methacrylated gellan gum were placed on the CAM at day 10 of embryonic development. Positive (filter paper or gelatin sponge with VEGF) and negative (filter paper or gelatin sponge) controls were also tested. The assay proceeded until day 14 or 18 of embryonic development and images were acquired *in ovo* and *ex ovo* using a stereomicroscope by the end of the assay. The images obtained were image-processed using the ImageJ program for facilitating the counting, which was performed by three independent observers. **Results:** The evaluation of the angiogenic response was performed by analysing the convergence of the blood vessels toward the implanted discs. Some degree of variability was found between replicates and inflammation occurred frequently, which hindered the analysis of the formation of new blood vessels. The reduction of the assay duration (from 18 to 14 days) resulted in a decrease of inflammation/contamination. All the materials were partially adsorbed during the assay. However, the controls didn't present a regular response and the gelatin sponge was often completely adsorbed. **Conclusions:** *In ovo* quantification method was more complex as compared to *ex ovo*. The results indicate that no differences exist between the hydrogels tested in what concerns to their angiogenic potential.

P224 (EI0300)

A Green Approach to Process Semi-Crystalline Natural-Based Polymers for Tissue Engineering Applications

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Objectives: Following the green chemistry philosophy, this work aims the design and development of new 3D architectures of natural-based polymers, combining ionic liquids (ILs) and supercritical fluid technology, with relevant applications in tissue engineering and regenerative medicine. With this purpose, SPCL, a polymeric blend of starch, which is one of the most abundantly occurring natural polymers and poly(e-caprolactone), a synthetic biodegradable polymer, was processed by supercritical fluid foaming, at different operating conditions. The use of this technique for processing natural-based polymers has been limited due to the fact that they are normally semi-crystalline polymers. This can be overcome by the use of ILs, which have recently been proposed as plasticizing agents of starch. **Methods:** The IL tested in this work was 1-butyl-3-methylimidazolium acetate and its plasticizing effect was demonstrated by the mechanical tests conducted. The production of porous and interconnected structures was carried out, hereafter, using CO2 as foaming agent. The effect of different operating variables, such as pressure, temperature and contact time on the porosity, interconnectivity and pore size distribution of the matrixes was evaluated and the morphology was analyzed by micro-computed tomography. **Results and discussion:** The results obtained suggest that the induction of porosity within the constructs depends largely on the diffusion of CO2 in the matrix, which explains the higher porosity of the samples processed at higher pressures and larger contact times. Moreover, the presence of IL has shown to have a key role in the success of the supercritical foaming process, and consequently on the preparation of porous and interconnected scaffolds. **Conclusions:** To our knowledge it's the first time that this approach has been reported. The findings described in this work can be extended and adapted to other raw materials, which largely broads the spectrum of natural-based polymers that may be processed into 3D porous matrixes.

P225 (EI0299)

PLLA-PEG Cryostructured Scaffolds Reinforced With Biodegradable Fibers

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Objectives: Poly(l-lactide) (PLLA), poly(glycolide) (PGA), poly(ethylene glycol) (PEG) and/or copolymers of these have extensively been used in medicine for applications. VICRYL is a commercially available suture produced from a copolymer of glycolide/lactide (90/10 mol/mol). In this study, Vicryl fibers were used for increasing mechanical properties of PLLA-PEG cryostructures. **Methods:** PLLA-PEG block copolymer was synthesized by ring opening polymerization of L-lactide dimer and PEG (6000 Da). Wet spun fibers were obtained from chitosan solution in acetic acid. Commercially available Vicryl was used as a source of Poly(lactide-co-glycolide) (PLGA) fibers. PLGA fibers with varying length (1, 2 and 4 mm) and matrix/fiber ratios (2:1, 3:1 and 4:1) were dispersed in % 5 (w/v) solutions of PLLA-PEG in 1,4 Dioxane. Porous scaffolds were prepared with these solutions under cryotropic conditions (–12 °C). **Results:** FTIR, and NMR spectra confirmed the chemical structure of PLLA–PEG copolymer. Cryostructures made from this copolymer had interconnected macropores which were obtained. They exhibited remarkable properties, including high flexibility and rapid size change to external forces, and also "swellability" in aqueous media. Vicryl fibers (both the amount and the fiber aspect ratio) increased mechanical strength of the PLLA-PEG cryostructures quite significantly. **Conclusions:** PLLA-PEG cryostructures reinforced with vicryl fibers were concluded to be a good candidate that can be used in tissue engineering applications for both hard and soft tissue regeneration.

P226 (EI0292)

Valorization of Valorization of Chitosan from Squid Pens and Further Use on the Development of Scaffolds for Biomedical Applications

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Objectives: The aim of the present work is the valorization of squid pens through the production of chitosan that can be used for the development of biomedical applications. The present work is focused on β-chitin extraction from squid pens of the species *Dosidicus gigas* and its further conversion into chitosan. The biomedical potential of the isolated squid chitosan was assessed by processing this polymer as scaffolds for tissue engineering strategies. **Methods:** Alkali solution was used to deproteinized squid pens and thus isolate β-chitin [1], which was further converted into chitosan through a deacetylation reaction. The chitosan scaffolds were developed using a freeze-drying process, from 3% and 4% chitosan solutions in acetic acid and freezing at temperatures –80°C and –196°C. Chitosan scaffolds were neutralized using two different methods: M1—NaHO solution; and M2—ethanol/water and NaHO solution [2]. Morphology, Mechanical properties, degradation, cytotoxicity (L929 cells) and cellular adhesion (ATDC5 Chondrocytes like cells) of squid chitosan scaffolds were assessed and compared with the properties of scaffolds produced with commercial chitosan. **Results:** The morphology of scaffolds revealed a lamellar structure for all produced scaffolds, independent of the origin and concentration of chitosan. The treatment with sodium hydroxide and ethanol caused the formation of larger pores and loose of some lamellar features. Different freezing temperatures gave different pore morphology and the lower temperature a smaller pore size. The *in vitro* cell culture and cell adhesion studies showed that all chitosan scaffolds exhibited a non-cytotoxic effect over the mouse fibroblast-like cell line, L929 cells. **Conclusions:** The chitosan produced from the endoskeletons of giant squid *Dosidicus Gigas* has proven to be a valuable alternative to the commercial one when considering its use as biomaterial for different biomedical applications.

P227 (EI0203)

Encapsulation of Human Mesenchymal Stem Cells Via Protein Cross Linking for Intervertebral Disc Regeneration

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Objectives: Low back pain is a common disease in modern society. One major reason is the degeneration of the nucleus pulposus (NP, part of the intervertebral disc). The regeneration of nucleus pulposus via cell therapy requires a biocompatible matrix which facilitates the differentiation of hMSCs (human mesenchymal stem cells) to nucleus pulposus cells. The enzyme transglutamin-

ase was found to be suitable to generate a gelatine matrix by cross-linking and could be utilized to immobilize hMSCs. Further natural ECM of the NP should be used for crosslinking and immobilization. **Methods:** The immobilized cells were cultivated for 21 days in media, containing growth and differentiation factors. Cells were seeded at a density of 4×10^6 cells per cm^3, equal to the density in the NP. Viability of the cells in the gelatine matrix was proofed by using a tetrazolium salt (WST-1). At certain dates RNA isolation was done following the phenol/guanidine isothiocyanate protocol. RNA was transformed into cDNA followed by a RT-PCR with a gel electrophoresis afterwards. Different primers were used to analyze the successful differentiation of hMSC-TERT into nucleus pulposus cells. In further analysis, NP extract isolated from pigs, has been added to the gelatine matrix. **Results:** The viability of the immobilized cells has been on a constant value over the differentiation period of 21 days. Thus the survival of hMSC-TERT in gelatine is proofed. The differentiation status of hMSC-TERT in the two different matrices (gelatine, NP extract) could be analyzed. **Conclusions:** Data concerning the differentiation of hMSC-TERT in nucleus pulposus cells in a gelatin matrix and differences between the two matrices will be presented.

Stents and Vascular Implants

P228 (EI0339)

Validation of a Test Setup for Haemocompatibility Testing of Small Cardiovascular Implants

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Objectives: Many of the parameters that have substantial influence on blood damage in *in vitro* haemocompatibility testing frequently remain undefined, leading to poor reproducibility. Taking into account previous research and prevailing lack of comparability, our goal is to develop a simple and reliable dynamic *in-vitro* test setup for testing small cardiovascular implants, such as stents. **Methods:** To keep dilution effects low, circulating blood volume was restricted to a minimum. As previous experiments have shown that roller pumps are not suitable for haemocompatibility-testing with small blood volumes, we used the well-known Chandler-loop mounted on a standard laboratory pump drive. All experiments were conducted with bare metal stents and heparinized porcine blood at 37°C. Three stents in a row were placed in polyurethane tuberings with an inner diameter of 2.4 mm. Haematocrit, haemolysis and platelet count were determined at 0, 1 and 4 hours. The tuberings then were rinsed with 0,5% Octoxinol 9 (Triton X) and reused to rerun experiments four times. **Results:** After 1 hour platelet count in tube rings containing stents had dropped by an average of 17% compared to reference tuberings while haematocrit and haemolysis remained unchanged. Similar results were obtained after 4 hours. The measured effect decreased after each rinsing procedure, but only for the tuberings containing stents. This indicates that stent surfaces underwent a change while in contact with blood that cannot be completely reversed by rinsing with Triton X. **Conclusions:** Unlike roller pumps, the Chandler-loop causes little initial damage to blood. The effect of bare metal stents on platelet count was clearly detectable after 1 hour, whereas rinsing diminished detectable differences. Experiments with surface-treated stents are currently in progress. The setup will also be used to test electrospun matrix components for vascular prostheses for haemocompatibility within a DFG-funded collaborative research centre.

P229 (EI0402)

Mechanical Characterization of Very Small Blood Vessels and Vascular Grafts

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Aims: The determination of biomechanical properties of small diameter vascular grafts and very small vessels is an important task. Apart from optimal adaptation of graft compliance to natural vessels, also biomechanical investigations are essential in frequently used animal models such as from rats and mice. In this work a method was established for testing mechanical behavior of very small vascular prostheses and natural vessels of less than 1 mm diameter by the use of a modified tensile testing machine. **Methods:** The mechanical behavior of electrospun vascular grafts and of murine thoracic aortae was measured. For this purpose a BOSE ElectroForce testbench system with a 200 N Linear motor (Bose Corp. MN, USA) was modified with a cantilever and probe fixation for ring-shaped specimens, which allows the application and measurement even of very small forces. Rings of 2 mm width were cut out of the aorta descendens. In the testing system, the rings were placed between two 0.2 mm pins and loaded in circumferential direction until rupture. Force—elongation curves were recorded. Maximum force, elongation and compliance in the physiological range were investigated. **Results:** For mice after 19 weeks high-fat diet (B6.129P2-ApoE/J, age: 254 ± 9 days), the test showed a maximum force of 0.41 ± 0.12 N. The strain at maximum force was 84.3 ± 15.5 %. Maximum forces and strains varied within the different zones of the aorta. The compliance at 100 mm Hg was calculated out of the measured data, using Laplace's law and was 36 ± 6.3 % / 100 mm Hg. **Conclusion:** The established method allows a reproducible and sensible measurement of mechanical properties in very small ring-shaped specimen of arteries and vascular prostheses.

P230 (EI0289)

Development of a Small-Caliber Autologous Vascular Graft "BIOTUBEs": Four-Year Animal Implantation

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Objective: Since ESAO 2005, we have reported that in vivo tissue-engineered autologous tubular tissues "BIOTUBEs" are useful as small-caliber vascular grafts in rat, rabbit and beagle dog models. Their autologous connective tissue walls (thickness: ca. 0.1 mm) exhibited rapid regeneration of hierarchical vascular wall structure. In this study, we report long-term implantation of Biotubes in rabbit and beagle dog models. **Methods:** Silicon rod molds (diameter: 2~5 mm, length: 20~50 mm) were placed into dorsal subcutaneous pouches of rabbits or beagle dogs. After 1 month, biotubes formed around the molds were auto-implanted to the respective animals. **Results:** In both kinds of animals, after removing the molds from the implants harvested with around connective tissue, biotubes mostly consisting of coarse autologous collagen fibers and fibroblasts were obtained. biotubes showed burst strength more than 1500 mm Hg. *Rabbit;* Total patency rate of 3 months is 81.8% (n = 11). Longest follow up is 2 years without any degenerative changes. Little thrombus was formed on the luminal surfaces completely covered with endothelial cells with parallel orientation to the direction of blood flow. With time, hierarchical arterial structures were reconstructed in the recipient body under hemodynamic conditions, including circumferentially oriented smooth muscle cells, collagen fibers bundles and elastin fibers. *Beagle dog;* Rapid tissue regeneration was observed in spite of the animal species. Angiography at 2 years (n = 1) and ultrasound tomography at 3.5 years (n = 1) and 4 years (n = 1) showed the graft patency, neither stenotic changes at anastomosis nor degenerative dilatation. **Conclusion:** Autologous biotubes with no synthetic support materials withstood systemic blood pressure and exhibited excellent performances as small caliber vascular prostheses for up to 4 years. Further studies with numbers are ongoing for the aim of future clinical applications.

P231 (EI0164)

The Core Laboratories for Investigation of Cardiovascular Explant Materials Heart Valve Prostheses and Stents Study

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Objectives: The Core Laboratories for investigation of failed cardiovascular implant materials, initiated by to UE grant COST Action 537, have been organized by Foundation of Cardiac Surgery Developed in Poland. *Objectives:* For FCSD, research centre working on heart prostheses, biomaterials and new surgery tools the goal is the improvement of medical devices in clinical practice from the analysis of implanted prostheses. The Physician, supported by scientific multidisciplinary team can recognize the reason of the surgical intervention and exchange the prostheses. **Methods:** The samples were harvested during routine explantation or substitution by other prostheses or during post mortem examination. The combined use of different methodologies for the explants analysis provided complementary information of both the material reaction to the biological environment and the host response to the implant. Our database consisted of the scanty clinical information, initial surface studies and histological studies. Following gross inspection and photographing, the devices were analyzed by optical (OM) and electron microscopy (SEM), Energy Dispersive x-ray Spectroscopy (EDX), Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR). Radiographic examination, microbiological analysis, biological evaluation of adsorbed proteins, and histological analysis were conducted on the materials and peri-implant tissues. Biological analysis included Electrophoresis and Western Blot for the evaluation of the proteins adsorbed on the different device components, and ELISA tests for specific protein quantification. **Results:** About 150 valves and 40 vessels prostheses and about 30 vascular stents have been stored & set up bank of explanted prostheses. The several kinds of valves prostheses damage has been modeled & tested. The few of our last unique results is the Edwards SAPIEN Transcatheter Heart Valve and heart coronary artery stent study. **Conclusions:** The report of achieving results and consideration about possibility for usage it for advisory system knowledge bank have been prepared.

P232 (EI0143)

Development of a Polyurethane Valved Conduit with Valsalva Sinuses for the Reconstruction of the Right Ventricular Outflow Tract (RVOT)

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Objectives: Around 20% of congenital heart defects require a surgical reconstruction of the right ventricular outflow tract (RVOT), often performed using valved conduits. Several studies have already proven the importance of Valsalva sinuses in such prostheses. Currently existing prostheses require permanent coagulation (mechanical prosthesis), have limited durability (xeno/homografts) and availability in small diameters. Thus, the aim of this work is to design and manufacture a complete prosthetic polymeric valved conduit with Valsalva sinuses. **Methods:** A two-part core which ensures sinus formation and valve integration into the conduit was manufactured. The conduit was then produced by polyurethane atomization (spraying), allowing the production of a fine-fibrous structure with a special micro-porous surface which can favour a neo-intimal layer formation. The micro-porous structure was observed and studied under microscopy. The prostheses' mechanical and structural properties—tensile strength, compliance, burst strength, permeability and suture retention—as well as the valve functionality were subsequently tested in vitro. **Results:** Laboratory samples with a 22 mm internal diameter were produced for in-vivo experiments in calves. Structure variations were applied through process parameters changes. Valve opening and closing was investigated with high speed video and functionality was assessed for pressure differences of 20, 40 and 80 mm Hg. The conduits showed sufficient compliance to propagate the pressure pulse and to withstand pressure peaks. The prostheses were impermeable up to 100 mm Hg pressure. The tensile, suture retention and burst strength were satisfactory. **Conclusion:** Production of valved conduits is possible using polymer atomization. In-vitro tests showed satisfactory results for mechanical properties and functionality. These results encourage the optimization and conduction of further research in this field. A chronic implantation of the conduits in calves is planned and results are expected August 2011. Further in-vivo experiments and in-vitro fatigue testing will be carried out to assess the conduits durability.

P233 (EI0144)

Improvement of Static Performances of Biomorphic Polymeric Heart Valve Prostheses by Tailoring the Material Orientation

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Objectives: Some styrenic block copolymers (SBC) having cylindrical morphology allow tailoring the microstructure to achieve anisotropic mechanical behaviour similar to natural tissues. The aim of this study is to investigate numerically the effects of material structure orientation on the performance of biomorphic symmetric trileaflet heart valve prostheses (HPV). **Methods:** Two oriented SBC (Kraton 19%wt-polystyrene from Kraton Polymers and V4111 19%wt-polystyrene from Dexco Corp.) were mechanically characterised. A hyperelastic anisotropic constitutive model was implemented into a commercial finite element code. A parametric CAD of a biomorphic symmetric HVP (size $22 \times 12 \times 0.35$ mm) in closed configuration was developed. Exploiting symmetry, a single leaflet was discretised into linear hexahedral elements. Uniform static pressure (180 mm Hg = 24 kPa) was applied. The code was equipped with an iterative algorithm for the reorientation of the fibres along the local maximum principal stress (MPS) direction, seeking for optimality conditions. The numerical data were analysed in terms of stress distribution, coaptation area (CA), and vertical leaflet slipping (VS), used as indexes of the HVP global performance. **Results and Discussion:** Kraton leaflets presents a more uniform stress state (MPS < 0.4 MPa for 60% of the elements). The maximum decrement of VS for this polymer is 16.4% with respect to the baseline calculation while V4111 displayed no significant changes. For both materials, maximum CA for optimised leaflets was 1% larger than the corresponding baseline, confirming that the coaptation mechanism is mainly driven by the flexural stiffness of the structure rather than by the tensile stiffness of the material. **Conclusions:** V4111 leaflet showed no significant enhancement of the mechanical and structural performances when optimised. Kraton leaflets showed a reduction of the VS at constant CA, denoting stabilisation of the closure of the valve. The study demonstrates that optimisation of the microstructural orientation of the material can enhance the performance of a polymeric HVP.

P234 (EI0226)

Fluid Dynamics Characterization of Regurgitant Flow in Mechanical Heart Valve

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Objectives: The regurgitant flow in mechanical heart valves has been frequently associated to blood trauma (hemolysis, platelet activation). In these devices, a certain amount of regurgitant flow is a desirable option, since it provides washout of the hinges, where blood particles, if trapped in low-velocity regions, could initiate aggregation phenomena and ultimately lead to thrombosis of the valve. Thus, regurgitation in mechanical valves is meant to maintain the valve surfaces clean of blood deposits; on the other hand, regurgitant-flow jets can have detrimental effects on blood, due to flow-related stresses. **Methods:** In order to characterize valve regurgitation, a physical model of the regurgitant flow, previously built at the ISS' premises, was used. The prosthetic valve under test was seated coaxially with a 12-face prism, enabling easy optical access. This model was inserted in a closed flow loop, in which the regurgitant steady flow is driven by 80 mm Hg transvalvular pressure, preset by positioning a reservoir at a suitable position above the valve plane. A Particle Image Velocimetry investigation was carried out on currently marketed bileaflet valves, by measuring the 2D flow field at several planes encompassing the plane of the two distal hinges. Mean and fluctuating velocities were calculated over 1000 instantaneous measurements. Maximum turbulence shear stresses (TSSmax) were also calculated, by means of Principal Stress Analysis. **Results:** The study enabled to reconstruct tomographically the jets exiting the valve. This information can be used as a reference standard for improving the assessment of valve function during echocardiography. Very low TSSmax values (not exceeding 12 Pa) were found, confirming the limited impact on blood of the tested valve. **Conclusions:** The experimental set-up provided an accurate characterization of the leakage phase, which has gathered increasing attention in the recent past, due to its potential contribution to thrombogenicity of cardiovascular implants.

P235 (EI0225)

Computer Aided Anatomical Fitting as a Method to Improve the Design of Cardiovascular Implants

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Objectives: Good integration of cardiovascular implants with the surrounding anatomy is an important implantation criterion. In this study the method of virtual fitting to optimize implant design was evaluated and compared to cadaver studies. The design of a Total Artificial Heart (TAH) was reviewed and optimized with regards to inlet and outlet orientation. **Methods:** Computer Tomography (CT) scans of 13 female and 10 male patients (ages 45–91) with cardiovascular disease were reconstructed into 3-dimensional geometries using a grey scale threshold and different segmentation algorithms as well as manual editing. Absolute location of all valves were identified and key parameters were calculated including valve diameter, orientation in global 3D space and relative distance to key markers in the thoracic cavity. The valve parameters were averaged to determine optimal inlet and outlet positioning for overall fit. The new CAD TAH geometry was superimposed CT geometries to verify volume fit within the anatomical space. Using models manufactured by rapid prototyping the new configuration was verified in seven cadaver studies. Additionally three of the cadaver anatomies were generated into computational geometries using a 3D- coordinate measuring system and compared to the CT data. **Results:** CT valve parameters varied between 13 % and 17% and were higher in male than female patients (M:15–23%, F:10–14%). The valve parameters measured during the cadaver studies were similar to those acquired in silico, however the variation was slightly higher (17–34%). The inlet and outlet configurations of the TAH chambers could be matched to the physiological values. **Conclusions:** Digital Fitting of cardiovascular implants is a powerful tool in the development of new devices. This method can not only be used for preliminary design optimization of medical devices thus reducing the number of cadaver studies, but also provides a method to examine fitting in anatomical environments unaltered by sternotomy.

P236 (EI0129)

Vascular Prostheses Manufactured by Spray-Atomization of Polymers

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Objectives: The use of porous materials for vascular prostheses can give some advantages over continuous surfaces. Porous surfaces encourage the formation of a thin neo-intimal layer onto the material, as has been shown by Karapinar et al.. Matching the mechanical properties of the graft with physiological values also benefits the acceptance of the graft in the body. It is therefore highly desirable to produce porous materials from biocompatible polymers. Our goal is to develop a manufacturing process for vascular grafts which produces thin fibers from a polymer solution, and has a higher throughput than electrospinning. **Methods:** A pneumatic spray gun is used to atomize a polymer-solution and to form small droplets which then dry and deform to fibers during their flight to the target. The nearly dry fibers then form a non-woven textile on the target. The target can rotate and the spray gun can move in two axes. Factors like atomization pressure, material flow, solvent, concentration and polymer used can also be varied. Sprayed samples are inspected by microscope and subjected to tensile tests. Sterilization tests have been conducted with gamma, ETO, plasma and steam sterilization. **Results:** It is possible to spray fibers with a diameter of 1 µm. The permeability of the textiles can be influenced by changing spraying parameters. Different polymers (PCU and PLA) have been successfully tested for this manufacturing process. The PLA can be used as a scaffold for tissue engineering, whereas the PCU can be used for polymeric graft prostheses. The PCU textiles showed good mechanical properties and a linear stress-strain behavior. The material can be sterilized by gamma, ETO and plasma sterilization. **Conclusions:** The spraying process shows promising results for the production of non-woven materials with properties beneficial to their application as grafts and scaffolds.

P237 (EI0210)

Decellularization of Bovine Pericardial Tissue for Tissue Engineering Applications May Alter Its Biomechanics and Tissue Components

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Objectives: We studied biomechanical behavior and biochemical composition of acellular bovine pericardium (BP) matrix, scoping to a scaffold capable of exhibiting the mechanical properties desired for tissue engineering (TE)applications, especially for treatment of cardiovascular system deficiencies. **Methods:** BP tissue was decellularized according to two different protocols: 1) Treatment based on Triton X-100 (12 hrs, 4°C). 2) A classic treatment with Trypsin/EDTA at 37°C for 48 h. Biomechanical viscoelastic characteristics, high modulus E_H, low modulus E_L and hysteresis ratio (h) were determined from dynamic cyclic tensile stress-strain testing (1 cycle/sec, saline wetted, 37°C). Biochemical determination of tissue components after papain digestion involved chondroitinase digestion for glycosaminoglycans followed by HPLC analysis and HCl hydrolysis for hydroxyprolin. **Results:** Biomechanical characteristics, E_H and E_L found to vary (40 to 50 MPa and 0.27 to 0.30 MPa respectively) in fresh untreated tissue, depending on anatomic direction. Decellularization by (1) had no mechanical effect (44.65 to 52.67 and 0.37 to 0.37 MPa) while after (2) a significant decrease was found (20.96 to 36.82 and 0.20 to 0.23 MPa). Hysteresis (h) ranged (19–26% of the loading energy dissipated), depended on anatomic orientation with no difference. Tissue glycosaminoglycans content was unaffected after treatment (1), while a 22% of chondroitin/dermatan sulfate and 60% of hyaluronan removed after (2). **Conclusions:** Decellularization of BP under long duration in 37°C (2) altered its biomechanical behavior, which seemed to be retained under low temperature short duration treatment (1). Decrease of glycosaminoglycans by treatment (2) may reduce biomaterial efficiency as TE scaffold regarding cell emigration, proliferation and function towards regenerative process. Cell-biomaterial interactions study is in progress.

P238 (EI0128)

Functioning Test of Vascular Smooth Muscle Cell Constructs

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Objectives: To analyse the efficiency and functionality of smooth muscle-like cell-(SMCl-) constructs, differentiated from adipose tissue derived stromal cells (ADSC) in an innovative test stand. **Methods:** ADSCs were differentiated into smooth muscle-like cells in different media (Medium A, B and C). ADSCs and primary SMCs were embedded in collagen-I gel-constructs. After installing the constructs in the testing device, force/time diagrams were recorded. The force generated by the constructs after application of NO, KCl or norepinephrine (NE) was measured. **Results:** SMCl-cells differentiated from ADSCs express, like primary SMCs isolated from human carotis, mRNA and proteins required for a functional cytoskeletal apparatus. Collagen-I gel-constructs could be obtained, which remained stable for at least 14 days, comprising cells of both origins. Application of NE or KCl to the culture medium in the test device resulted in the spontaneous contraction of the constructs. Relaxation of the cell-constructs could be observed when the culture medium was supplemented with NO. The extend of the induced constriction or dilation was highest in constructs made of primary, terminally differentiated SMCs. SMCl cells differentiated in medium C exerted higher forces than cells derived from medium A or B. **Conclusions:** Patient derived adipose tissue derived stromal cells can differentiate towards smooth muscle-like cells in vitro. For the application in a tissue engineered vascular prosthesis the differentiated cells have to prove functionality. With this test stand we are able to analyse the efficacy of contractile smooth muscle differentiation.

P239 (EI0198)

Laser Assisted Bioprinting for the Generation of Vascular-Like Structures

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Objectives: To generate vascular-like structures by laser-assisted bioprinting (LaBP) for the application in tissue engineered constructs. **Methods:** LaBP was applied to seed alternating spots of human endothelial cells (ECFCs) and human adipose tissue derived stromal cells (ADSCs) in vicinity to each other on a planar recipient plate. The distance between the cell spots was set to 600 µm. Both cell types were embedded in a fibrinogen-hyaluronic-acid hydrogel which was consolidated by a subsequent treatment with thrombin. The printed recipient plate was incubated in endothelial growth medium without the supplementation of VEGF for 10 days. Images were taken every day in order to monitor the development. **Results:** First, ADSCs started to grow towards the direction of the nearest spot of ECFCs which remained quiescent during the first days of incubation. At day 3 ADSCs contacted the ECFCs which in turn also proliferated and participated in the formation of vascular-like structures. These structures remained stable for the incubation period of 14 days. Furthermore, we could show that LaBP is also capable of printing stacked levels of cell spots in order to create three-dimensional constructs. **Conclusions:** LaBP printing of ECFCs and ADSCs in direct vicinity to each other leads to the formation of vascular-like structures. Combined with the possibility of printing in three-dimensions, this technique might be a useful tool for the reproducible generation of tissue constructs with a pre-defined capillary bed.

P240 (EI0119)

Creation of Devitalized Vascular Prostheses of Small Diameter

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Objectives: Existing methods of prosthetic repair in aortal-coronary bypass have some serious short-comings. There is a deficit of autological prostheses, their isolation traumaticity increases the risk of infectious and inflammatory complications. Allogeneic prostheses are inclined to rejections and thrombosis. Synthetic prostheses of small diameter are not suitable for such a prosthetic repair. The technology of creation of biological prostheses based on xenogeneic vessels has not been effective till now. This study conception is based on the use of two physical factors: low temperatures and irradiation with electrons to devitalize the xenogeneic arteries with preservation of their strength properties. **Methods:** The research object was mature porcine intrathoracic arteries, isolated with meeting the bioethical protocols. Procured vessels were subjected to freezing down to –196°C and irradiation with electrons in the experimental doses. Biomechanical properties were examined with strength- and burst-tests, as well plasticity was estimated. There were performed histological studies of vascular scaffolds, including those after xenogenous transplantation under skin and into system blood flow as vascular prosthesis. **Results:** After freezing of the vessels there were noted vast sites of endothelium desquamation. The following irradiation with electrons causes a complete devitalization of arteries. Structural integrity of connective tissue fibers of vascular wall after irradiation was not impaired. The endurance of arteries in longitudinal direction increased after freezing. When measuring the durability in radial direction there was found its rise in the group of irradiated arteries if compared with native vessels. No acute inflammations were found after implantation of the treated arteries under the skin of rats for all the observation terms. The adequate functioning of the treated vessels was shown during implantation into system blood flow. **Conclusions:** The proposed devitalization method allows the creation of integrally functioning biological vascular prostheses and may be an alternative when selecting the grafts for aortal-coronary bypasses.

P241 (EI0116)

Six-Month Auto-Implantation of Autologous Tissue Small-Caliber Vascular Grafts, "Biotube", to Carotid Arteries of the Beagle Dogs

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Objectives: We have reported that in vivo tissue-engineered autologous tubular tissues "biotubes" could be ideal small-caliber vascular grafts in the animal experiments. They are constructed in the recipient bodies safely and economically without any use of special clean facilities. In this study, biotubes were auto-implanted to the carotid arteries of the beagle dogs, and histological change of biotubes after implantation was evaluated. **Methods:** Biotubes were prepared by placing silicone rods (outer diameter, 5.0 mm; length 80 mm) used as a mold into dorsal subcutaneous pouches in the beagle dogs for 4 weeks. The obtained biotubes after nonthrombogenic drug coating were auto-implanted to carotid arteries of the same beagle dogs by end-to-side anastomosis using 6-0 nylon continuous suture. Graft status was evaluated by digital subtraction angiography. Histological evaluation was performed 3 and 6 months after implantation. **Results:** After 4-week preparation in the subcutaneous tissues, the molds were completely covered with autologous connective tissues (thickness: ca. 0.1 mm) mainly consisting of fibroblasts and collagen fibers. During 6-month implantation, neither formation of aneurysms nor rupturing was observed in biotubes. On the luminal surfaces, endothelial cells were covered partially in the vicinity of the anastomotic region of the grafts at 3 months after implantation. On the other hand, endothelial lining was formed completely at 6 months. **Conclusions:** Biotubes could be used as small-caliber vascular prostheses that greatly facilitate healing process and exhibit excellent biocompatibility in vascular regenerative medicine.

P242 (EI0075)

Application of CAD/CAE-Technologies for Working Out of a Universal Design of an Artificial Ventricle of Heart

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Objectives: Working out of a universal design of an artificial ventricle of heart (AVH) for devices of auxiliary blood circulation with an electromechanical and pneumatic drive for application in экстракорпоральных и implanted systems. **Methods:** For the decision of the given problem methods of computer modeling were applied to definition of geometrical parameters of AVH and methods of the certainly-element analysis for a finding of optimum hemodynamic parameters of work of AVH. Geometrical modeling was spent in system Pro/Engineer WF 4. System Ansys was applied to the analysis of a current of fluid in chamber of AVH. Modeling was spent with both a moving, and a stationary membrane with the task of beginning and boundary conditions. The system was put to the test on the test bench for definition of an output and an imaging of a current of fluid in chamber of AVH by application the laser set, which works by cooper steam, model CVL-10 (λ = 510 nm, Russia), and high speed camera, model CCD—SMOS VS—FAST (speed of shooting 5000 fps, Russia). **Results:** Hemodynamics key parameters, giving the chance to optimize a design by hemodynamic indicators are defined. **Conclusions:** The model of optimum design of AVH for application in considered designs is received.

P243 (EI0048)

Development of Construction Techniques for Tissue Engineered Heart Valve Substitutes

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Objectives: Among the four heart valves, the aortic valve is the one exposed to the most severe flow and pressure conditions, making it more prone to diseases such as stenosis or regurgitation. In order to reduce risks of cardiac insufficiency, diseased valves need to be replaced. Mechanical or tissue valves are often used despite their major disadvantages related to increased risks of immunological response, limited life expectancy and lifetime anticoagulation therapy. On the other hand, tissue engineering offers the possibility to create biological substitutes with the potential to adapt and grow with the surrounding tissues, hence eliminating the need for lifelong medication and risky subsequent surgery. **Methods:** A detailed review of the literature allowed us to identify the construction techniques with the most potential. Two methods will be developed. The first one will use a natural scaffold made from decellularized porcine valve from which cells will be removed by chemical, enzymatic and mechanical techniques. Dynamic seeding will follow in a custom made bioreactor designed by our team. In the second construction technique, the tridimensional complex structure of the aortic valve will be reproduced by a manufacturing process using purpose built tools and tissue sheets made with the self-assembly method. **Results:** Tissue sheets culture followed by preliminary tests confirmed the feasibility of heart valve construction based on two-dimensional materials. However, further tests will be necessary in order to determine the required thickness of the sheets and then optimizing the developed techniques. Simultaneously, decellularization protocol of the porcine scaffold was established. First tests using this method will be performed in the next months. **Conclusions:** A detailed literature review on heart valve replacement technologies as well as preliminary tests performed by our team validated that tissue engineering techniques can lead to the development of entirely autologous heart valve substitutes.

P244 (EI0008)

Development of Microporous Self-Expanding Stent Grafts for Treating Cerebral Aneurysms—Designing Micropores to Control Intimal Hyperplasia

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Objectives: Treatment of large cerebral aneurysms with a broad neck in the cranio-cervical area is difficult and carries relatively high risks, even with surgical and/or endovascular methods. To this end, we have been developing a high-performance, self-expanding stent graft. **Methods and Results:** A commercially available NiT stent (5 mm in diameter, 20 mm in length) was covered with a thin microporous segmented polyurethane membrane fabricated by the dip-coating method, then micropores were created by the excimer laser ablation technique, and the outer surface was coated with argatroban. Micropores with two patterns were designed. One type had a circular shape (diameter, 100 μm; opening ratio, 12.6%), and the other was bale shape (size, 100 × 268 μm; opening ratio, 23.6%). Experimentally fabricated side-wall aneurysms of both canine carotid arteries using venous pouches from external jugular veins were placed with the self-expanding stent graft on one side, and with a bare self-expanding stent on the other side. All carotid arteries were patent and free of marked stenosis after 1 month. All aneurysms were occluded by stent grafts, while patent in those treated with bare stents. Histologically, the stent grafts with bale-shaped micropores and a high opening ratio were associated with less intimal hyperplasia (187 ± 98 mm) than bare stents (341 ± 146 μm) or the stent grafts with circular micropores and a low opening ratio (441 ± 129 μm). A pore ratio of 23.6% was found to control intimal growth. **Conclusions:** Our self-expanding stent grafts were easily applied to experimental aneurysms, and had achieved complete aneurysm occlusion in beagles at 1 month after implantation. The stent graft with high opening ratio was associated with less intimal hyperplasia than a bare stent or a stent graft with low opening ratio.

Robotics and Instrumentations

P245 (EI0383)

Ultrasound Lung Comets in Patients Undergoing Hemodialysis

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Objectives: Sonographic B-lines (also called ultrasound lung comets—ULCs) are a sign of extravascular lung water (EVLW). Absent in normal lungs, B-lines rise up as the lung become congested and the interstitium starts to fill with fluid because sound is transmitted through these fluid-filled spaces and reflected between the walls of the congested interstitium. ULCs appear as hyperechogenic bundle with narrow basis spreading from the transducers to the further border of the screen. Aim of this study was to demonstrate the utility and easiness of chest ultrasound to evaluate lung congestion in hemodialysis. **Methods:** Ten patients underwent two chest ultrasound examinations: before and after dialysis. We followed a chest ultrasound protocol that counts the number of ULCs visualized in 28 lung zones. For each patient were recorded baseline demographics, Bioelectrical impedance vector analysis (BIVA), pulse oximetry, thoracic physical examination pre and post dialysis, volume of fluid removed, subjective dyspnoea. **Results:** All patients had initial predialysis ULCs > 14. After dialysis, seven of ten patients had <14 ULCs. Pre-dialysis ULCs medium score was 34.4 ± 23.5, post-dialysis medium score was 10.7 ± 7.2. Medium volume removed was 2.5 L. At the post-dialysis time point, for every 500 mL volume removed, there was a decrease of 4,7 ULCs. Nowadays methods to evaluate dry weight in patients undergoing dialysis are based on blood pressure, BIVA, presence of oedema and thoracic physical examination. Our data, even if in a little group of patients, support chest ultrasound as an easy, low cost, useful method for evaluating real-time changes in EVLW in patients undergoing dialysis, often highly congested. **Conclusions:** Chest ultrasound is an easy and low cost method, useful and less harmful than x ray, to evaluate lung congestion in patients undergoing dialysis.

P246 (EI0171)

Surgery Telemanipulator Steering Console as Integrated Biomedical Data Processing Centre in Man-Machine Interface Optimization

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Objectives: Well known advantages of minimally invasive surgery (MIS), can be strengthened using telemanipulation systems for surgery support. Movement scaling, precision, surgeon ergonomy improvement especially during long-term MIS procedures are features of the surgery robotic systems, where surgeon manipulate sitting next to steering console with haptic input device (master) to control the remote (slave) manipulator with camera or tool. The goal of presented work was to optimize this Man-Machine Interface, developed in the the project of RobIn Heart telesurgery system. In assumed approach the Master Console—⟨⟨RHShell⟩⟩ was treated as a integrated centre of two main data channels: optical channel with 3D visualization and main master-slave control system, which maps surgeon hand movement with force feedback pilot study. **Methods:** Developed master-slave bilateral control system, transfers scaled and filtered human operator commands from 18 degrees of freedom (DOF) of master handle to 3 slave arms (2 with surgery tools and 1 for camera channel—total 16 DOFs). It was implemented on the effective field programmable gate array (FPGA) structures, which allow to build own system on chip with very high data processing, direct on silicon. Visual feedback channel, provided to surgeon in 3D Master console was innovatively developed in the project using two separated optic channels with empirically chosen distance and angle. **Results:** Ergonomy and control effectiveness in real and virtual environment was tested on four different type Man-Machine interface—space manipulator with spherical structure, foot multifunction unit, medical joystick and manual pilot. Position, velocity and torque mode of control system was implemented and tested during telesurgery on pig organs (in vitro) and two animal experiments (in vivo). **Conclusions:** Innovations, introduced to impove the Master console have a crucial meaning for whole surgery robotic system, which decides of its acceptance in medical world.

P247 (EI0165)

Artificial Tissue Mechanical Model as a Standard Object for Surgical Tools and Robots Testing

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Objectives: Tissue analogs are known area of research in the field of artificial organs. The ethical and scientific reasons, is going for tissue models of some properties (mechanical, electrochemical) is a wide field of applications. There is also a need for unified model of tissue for studies of new surgical instruments. **Methods:** Several tissues of the cardiovascular system, describes its mechanical properties have been examined. On the other hand, common tasks, features a tool-tissue contact (touch, capturing tanks traumatic, atraumatic, tissue cutting, separation, etc.) have been developed. **Results:** In order to standardize the testing of innovative tools Artificial Tissue interaction electromechanical ATM model have been developed. Appropriately programmed computer controlled electromechanical system have been designed and executed. Algorithms mimic (pretending) determined tissue-tools of achieved contact have been developed. The classical instruments, laparoscopic, its own prototype tools: mechatronic and robotic Robin Heart surgical instruments have been studied on ATM model. **Conclusions:** A unified, repeatable research station with significant opportunities to pretend to react with natural tissue have been achieved. The ATM station is currently being used in the research of innovative surgical instruments developed at the Laboratory of Biocybernetics FCSD.

P248 (EI0259)

Robot as an Artificial Surgeons Organ for Mini-Invasive Operation. From the Analysis of the Work the Surgeon's Hand to the Robotic Arm Design Robin Heart

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Introduction: The surgery success depend on the information, knowledge, experience and capabilities of surgeon precision motion. Leaving in the hands and brain of the surgeon in guiding and deciding telemanipulator are examples of artificial organs dedicated to specific tasks. Miniinvasive surgery provides poorer, less intrusive information, often only visual observation of the field operations. Telemanipulators thanks to that between the hand surgeon and a tool inside a patient's body is a computer—you can create a technology and technical methods and decision support systems to improve precision for the task (removing tremors hand, scaling of movement). Objectives: This approach to design innovative instruments introduced in the Polish surgical robot, Robin Heart, project. **Methods:** The process of developing the robot starts from the determination of the tools- tissue reaction (mechanical characteristic, the forces for specific operations, dynamic analysis of tools work) and person—a tool and then man-machine contact (kinematic analysis of surgeon motion). Kinematics values of multibody system: surgeon's upper limb—the classic and thoracoscopy tool or master tool manipulator—robot in different configuration (for typical surgery action elements), were determined with the use of APAS system for the trajectory of marker observation, recording and analyzing. On this basis, some typical tasks, functions, tools, and the operator behavior have been listed and a surgical robot control console trying to optimize both the issues of ergonomics, accuracy and .. work have been designed. Robin Heart Shell console includes not only ergonomic handles with microjoystics but also the advisory system and the possibility of a full visual and voice communication with the operating theater. **Results:** The ergonomic and high efficiency useful Robin Heart robot system have been constructed and successfully tested in few animal and telerobotic experiments.

P249 (EI0017)

Development of Haptic Perception Device Using "Touch Blend"

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Objectives: Surgical robots, artificial arms, and artificial legs are based on the research to present a sense of touch (haptic sense). Various researches to express a sense of touch objectively have been carried out. These have ever presented a portion of haptic perception of a sense of pressure, and of gravity. However, it is hard to say that they could express what a complex sense of touch information. **Methods:** In the 1910's, E.B.Titchener proposed "Touch Blend" of mixed haptic elements. Based on this idea, he proposed also "Touch Pyramid" of haptic sensations, which used a sense of pressure, of vibration, of warmth and cold, and of pain to express how sense of touch percepts. But it had a lot of ambiguous definitions of human haptic receptors and "Touch Pyramid". Then, it needed to examine again the ambiguous points of "Touch Blend". According to these examined results, a simplified haptic perception device was produced using a vibration motor, a peltier device, and a pressure sensor. This could choose three senses among a sense of pressure, of vibration, of strain, of warmth, of cold, and of first pain. Senses of touch were analyzed when haptic perceptions were presented on the tip of a finger using this device. Thirty persons of 20's were to answer a prepared list. To avoid the sense of sight, the experiments were carried out touching the device in a black-box. **Results:** As results, the device was able to present the six element senses. And it was also able to present some haptic perceptions of heat, of titillate, and of numb. There remained some difficulties to present other complex sense of touch informations completely. **Conclusions:** This basic research showed our device will be useful in the surgical robots, the artificial arms, and the artificial legs.

Biofluid Mechanics

P250 (EI0374)

Experimental Anaysis and Computer Simulation of Plaque Formation

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Objectives: Inflammatory process starts with penetration of low density lipoproteins (LDL) in the intima. This penetration, if too high, is followed by leukocyte recruitment in the intima. This process may participate in formation of the fatty streak, the initial lesion of atherosclerosis and then in formation of a plaque. Quantification of parameters for LDL transport and plaque formation is a huge challenge for the medical community. **Methods:** In this study, experimental model of LDL transport on the isolated blood vessel from rabbit on high fat diet after 8 weeks is simulated numerically by using a specific model and histological data. The 3D blood flow is governed by the Navier-Stokes equations, together with the continuity equation. Mass transfer within the blood lumen and through the arterial wall is coupled with the blood flow by the convection-diffusion equation. LDL transport in lumen of the vessel is described by Kedem-Katchalsky equations. The inflammatory process is solved using three additional reaction-diffusion partial differential equations. All parameters for computer model were fitted with nonlinear least square procedure. **Results:** Matching of histological rabbit data is performed using 3D histological image reconstruction and 3D deformation of elastic body. Concentration of macrophages obtained by computer simulation indicates that there is a newly formed matter in the intima, especially in the 5 *mm* region before bifurcation. Computed concentrations of labeled LDL of 13 % are in good agreement with experimental results. **Conclusions:** Matching of labeled LDL and macrophages concentration and location between experimental and com-

puter model shows a potential benefit for future prediction of this complex process using computer modeling.

P251 (EI0296)

Safety and Efficacy Study of a Novel High Vacuum Chest Drainage System

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Objectives: To assess safety and efficacy of a novel high vacuum chest drainage system (HVCDS) compared to a conventional chest drainage system (CCDS). **Methods:** Four anesthetized 40 kg pigs underwent a median sternotomy. Three drains were placed in retro-cardiac, retro-sternal and left pleural positions. Two animals received HVCDS (22Fr with 180 2mm-holes; Medela AG, CH), the remaining CCDS (28Fr-Atrium). After chest closure animals had three 30-min runs of artificial bleeding by infusion of citrate blood (4 ml/min) under different aspiration pressures (2,20,40 kPa) for both groups, followed by a standardized surgical bleeding (40kPa-HVCDS, 2kPa-CCDS). The output of all drains as well hemodynamic parameters were registered every 5 minutes. The amount of residual blood was also measured. After euthanasia all drains, the heart and left lung underwent macroscopic and/or histo-pathological examination. **Results:** The application of the different pressures showed neither hemodynamic changes nor differences in blood drainage with both systems during blood infusion and surgical bleeding. HVCDS had a trend to lower residual post-drainage blood:10(0–15) ml vs. 16(5–25) ml (p = 0,051). However, all HVCDS catheters showed relevant clotting. Application of 20 kPa and 40 kPa caused macroscopic epicardial and pulmonary lesions (chest tube holes) in both systems. Subepicardial or subpleural haemorrhage (depth 0.7 mm) without myocyte or alveolar damage was found by histology. **Conclusions:** Both systems showed adequate equal drainage, despite marked chest tube clotting in HVCDS. High pressure drainage with both systems showed focal subepicardial and subpleural bleeding. Thus, long-term assessment of high pressure chest drainage and potential interaction with fragile structure such as CABG graft should be carried out.

P252 (EI0256)

Performance Evaluation of Rheo-Microscope Image Processing Software for the Study of Erythrocyte Aggregation

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Objectives: Aggregation properties of red blood cells (RBCs) play a key role in the circulation, contributing to the hemorheological profile. In this study we have evaluated original image processing software for studying RBCs aggregation, in view of improving the characterization of blood diseases associated with hemorheological abnormalities. **Methods:** Whole blood was subjected to increasing shear rates (SR = 1 to 250 s^{-1}) at 37°C and simultaneously imaged with a rheo-microscope (Anton Paar), comprising a parallel-plate rheometer and an optical microscope (20×). A video sequence of the flowing erythrocytes was acquired during each test, and subsequently analyzed. A custom software for image segmentation, previously designed and validated by the authors, was used to determine, for each frame, the number and size of RBC clusters. **Results:** Normal RBCs at rest or under low flow can be found as aggregates. An increase in SR causes their progressive fragmentation. The software tracked the total area of the aggregates, which can drift along the experiment and must be normalized by the cluster number. At moderate SR, the number of new RBC clusters per unit of time (dN/dt) was seen to increase, from 0.66 clusters/s at 50 s^{-1}, to 1.18 clusters/s at 100 s^{-1}. Correspondingly, the temporal gradient of mean cluster area (dA_{mean}/dt) was –26.48 $\mu m^2/s$ and –25.13 $\mu m^2/s$, respectively (i.e., approximately 1 RBC lost every 2 seconds). Actually, dN/dt and $d(A_{mean})/dt$ have an opposite theoretical dependence on the number of clusters (N), whereas their product's absolute value is weakly dependent on N, and instead increases with SR, exactly as hereby observed. Finally, complete disaggregation occurred at SR = 200 s^{-1}. **Conclusions:** Image analysis of RBCs was found to be capable to track automatically erythrocytes' aggregation properties. Direct calculation of geometric features of RBC clusters is feasible, without resorting to indirect measurements such as, e.g., those based on back-scattered light.

P253 (EI0195)

Passive Flow Regulation in Capillary Networks

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Objectives: Capillary blood flow is a two phase flow: blood cells and plasma are moving through a network. What forces shape this flow? How are the cells distributed? What are the regulation mechanisms? This is investigated experimentally with an enlarged model, which is made to simulate the deformability of the red blood cells and the resulting effects on flow resistance. This experimental model was developed because in experiments with real blood the network is too minute and detailed numerical simulation is too complex. **Methods:** We use a blood model that consists of water droplets of about 1 mm diameter (red blood cells) and sunflower oil (plasma). Two syringe pumps pump the components into a bifurcation, where the droplets are formed. The model is applied for flow through bifurcations and through a network. The network model is made of transparent silicone rubber. It consists of capillary segments, whose diameters and lengths are statistically similar to the pulmonary capillary network of rats, but enlarged to an average diameter of 1.2 mm. **Results:** The experiments in the capillary network model show a droplet distribution which is variable, especially in segments with lower droplet fraction. A segment holds only a small number of droplets. Hence, a segmental flow resistance changes considerably when a droplet enters it or leaves it. Consequently, the flow distribution changes, which acts back on the number of droplets entering the segment—an unsteady flow is induced. **Conclusions:** Effects seen in both systems—in vivo and in the model—must be essentially influenced by the properties they have in common. This means, the variable hematocrit seen in pulmonary networks is at least in part a passive mechanism, induced by the flow resistance change due to the red blood cells and the droplets, respectively.

P254 (EI0110)

A Computational Model of Pulsatile Flow Past an Oscillating Cylinder

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Objectives: A fundamental study to characterize the flow around an oscillating cylinder in a pulsatile flow environment is investigated. This work is motivated by a new proposed design of the Total Artificial Lung (TAL), which is envisioned to provide better gas exchange. **Methods:** Cylinder oscillations and pulsatile free stream velocity were represented by two sinusoidal waves with amplitudes A and B and frequencies ω_c and ω, respectively. The average free stream velocity is U_0. A Newtonian fluid was considered and the governing Navier-Stokes equation was solved using the finite volume method. **Results:** It was observed that an increase in amplitude and a decrease in The Keulegan-Carpenter number ($K_c = U_o/D\omega_c$) are associated with an increase in vorticity (up to 246%) for every Reynolds number ($5 < Re < 20$) suggesting that mixing could be enhanced by the proposed TAL design. The drag coefficient was found to decrease for higher amplitudes and lower K_C for all cases investigated. In some cases the drag coefficient values were found to be lower than the stationary cylinder values ($A = 0.5, K_c = 0.3$ and $Re = 10$ & 20). A "*lock-in*" phenomena (cylinder oscillating frequency matched the vortex shedding frequency) was found when $K_C = 1$ for all cases. This lock-in condition was attributed to be the cause of the rise in drag observed in that operating regime. **Conclusions:** The results suggest that operating the TAL at higher fiber oscillation amplitudes and lower K_C (avoiding the lock-in regime) may provide optimal performance of the modified TAL design.

General

P255 (EI0361)

Application of Multiwalled Carbon Nanotubes in Thermal Treament of Cancer

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Objectives: To investigate the ability of multi walled carbon nanotubes (MWCNT) in absorbing the near infrared light for the treatment of cancer. **Methods:** Colorectal cancer SW620 and HT29 were seeded into two separate 96 well plates for 24 hrs. A group of cells were then incubated with variable concentrations of functionalised MWCNT (0.1 mg/ml, 0.2 mg/ml and 0.3 mg/ml) for 24 hrs. The wells were then exposed to 808 nm laser light with power of 1 W/Cm^2 at different time intervals of (1, 2, 3, 4 and 5 minutes). Cell viability for cells with MWCNT, laser or in combination was measured and compared with cells without MWCNT and laser, using variable techniques such as Alamar blue, Trypan blue and Fluorescence Activated Cell Sorting (FACS). **Results:** Result suggest that in the group of cells that were treated with both laser and MWCNT, by increasing the concentration of MWCNT and the timing of exposing the laser to the camcer cell, the number of cell death significantly increase. The cells that were treated with MWCNT without laser maintained high cell viability, similar to untreated sample. The results also show that in the case of laser alone, by increasing the duration of exposure of laser on the cell the number of cell death increase. However this is occurring with a lower magnitude in comparison to the combination of laser and the MWCNT. **Conclusions:** MWCNT has the ability of absorbing the near infrared wavelength. This property of MWCNT can be used for the thermal treatment of cancer.

P256 (EI0298)

Biological Tissue and Metal Material Adhesion Technology Using Integrated Low-Level Energies

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Objectives: We have proposed a new adhesion method by using integrated low-level energy sources. This adhesion method is expected to be applied to adhere biological tissue to the metal, such as stent-grafts, synthetic blood vessel and blood inlet cannula of ventricular assist devices. As fundamental research to adhere biological tissue to biocompatible metal material, we examined the relationship between adhesion performance and surface roughness of metal material. **Methods:** Shear tensile tests on slices of porcine aorta adhered to a metal specimen were performed to determine the relationship between adhesive strength and surface roughness of the metal specimen. The metal materials used for specimen was stainless steel which are used extensively as biocompatible material. The surface of metal specimen was roughened up with sandblast. The roughness of original surface metal specimen was an Ra of 0.05 µm. Surface roughness of sandblasted metal specimens ranged from an Ra of 0.25 µm to an Ra of 0.80 µm. **Results:** Biological specimen can be adhered to metal specimen. The adhesive strength of the biological specimen to the original metal specimen at an Ra of 0.05 µm was 0.25 MPa, and is higher than aldehyde glue adhesive strength that is 0.01 MPa. The average strength of surface roughens specimens ranged from 0.35 MPa to 0.45 MPa and is higher than that of the smooth original specimen. **Conclusions:** We proposed a new method to adhere biological tissue to metal material using integrated low-level energy sources. The adhesive performance was found to be improved by roughening the surface of the metal specimen. Our adhesion method is less invasive, has a more powerful adhesive strength than aldehyde glue.

P257 (EI0118)

A New Method to Quantify Eye-Blink Restoration in Facial Paralysis

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Objectives: Electrical stimulation (ES) of the paralyzed eyelid, triggered by the corresponding function on the contralateral healthy side, has been proposed to treat eyelid closure impairment in unilateral facial paralysis. However, results in terms of functional and cosmetically acceptable eyelid closure have been poorly documented. We propose a new method to quantify the effective restoration of the blink provided by a prototypal device developed for a contralaterally elicited eyelid ES. **Methods:** On a healthy subject surface EMG electrodes were used to detect the natural eye-blink (N-blink) on one side and to trigger an electrical stimulation leading to an artificial blink (A-blink) of the orbicularis oculi muscle on the controlateral side. Trains of 10 pulses (4 mA amplitude, 2 ms duration) were delivered at various frequencies: 50, 100, 150, 250 Hz. Assuming that during the eye-blink the eyelid rotates around the axis passing through eye canthi, a miniature gyroscopic sensor was used to estimate maximum eyelid motion from open eye to complete closure (CC) and eye-blink duration (D). Both the N-blink and A-blinks evoked by the different patterns of stimuli were measured and compared. **Results:** The N-blink was characterized by a CC of 3.5 mm and D of 380 ms. Stimulation at 50 and 100 Hz showed distinct differences from the N-blink: 50 Hz train caused ineffective eyelid closure (CC = –41%) and an excessive eye-blink duration (D = +215%), while 100 Hz train showed a more complete eyelid closure (CC = +6%) but longer eye-blink duration (D = +67%). Conversely, 150 Hz train and 250 Hz train provided a quite natural-like A-blinks (CC = +10%,+11%; D = +14%, –15%, respectively). **Conclusions:** The new gyroscopic-based method showed to be a valuable tool to quantify the effective and natural-like eye-blink restoration provided by the EMG–triggered ES device. Further studies will be necessary to evaluate the method in facial paralysis patients and to also provide quantification of potential alterations of facial mimicry associated with ES.

P258 (EI0105)

Liposome-Encapsulated Hemoglobin Enhances Chemotherapy to Suppress Tumor Growth and Metastasis in Mice

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Objectives: Liposome-encapsulated hemoglobin (LEH) with high O_2-affinity ($P_{50}O_2$ = 10 mm Hg, h-LEH) has been reported to enhance tumor radiosensitivity. Anticancer drugs, such as doxorubicin (DXR) but not 5-FU, require O_2 to be cytotoxic as in radiotherapy. We hypothesize that targeted O_2 delivery to tumor hypoxia by h-LEH may enhance chemotherapy and evaluated effects of h-LEH added to chemotherapy on tumor growth and metastasis in mice. **Methods:** DXR and S-1 (a novel oral 5-FU derivative) were applied on the Lewis Lung Carcinoma (LLC) grown in the leg of C57BL/6N mice. Daily administration of DXR (0.5 mg/kg, intraperitoneally) or S-1 (8 mg/kg, orally) was started 48 h after intramuscular inoculation of LLC for 2 consecutive weeks. H-LEH (5 mL/kg) was intravenously infused 2 h after each chemotherapy every other day for 2 weeks. After these 2 W treatments, mice were sacrificed for quantitative and macroscopical examinations of the tumor growth and lung metastasis. **Results:** Administration of h-LEH (5 mL/kg) or DXR (0.5 mg/kg) alone had no effect on tumor growth in the leg and metastasis in the lung. Addition of h-LEH to DXR resulted in 30.5% reduction of tumor weight ($P < 0.05$) and 41% reduction of lung metastasis ($P < 0.01$). While S-1 had a marked effect on both tumor growth (35% tumor weight reduction) and 62% reduction of metastasis, addition of h-LEH had no synergistic effect on the anti-tumor effect of S-1. **Conclusions:** These results suggest that h-LEH may be effective to enhance chemotherapeutic agents that require tumor oxygenation to suppress tumor growth and lung metastasis in mice.