

A COMPUTATIONAL MODEL OF CHEMICAL AND MECHANICAL PLATELET ACTIVATION AND AGGREGATION

*Original*

A COMPUTATIONAL MODEL OF CHEMICAL AND MECHANICAL PLATELET ACTIVATION AND AGGREGATION / Cardillo, Giulia; Biasetti, Jacopo; Cornat, Francois; Barakat, Abdul. - In: INTERNATIONAL JOURNAL OF ARTIFICIAL ORGANS. - ISSN 0391-3988. - ELETTRONICO. - 42:8(2019), pp. 409-409. [10.1177/0391398819860985]

*Availability:*

This version is available at: 11583/2812832 since: 2020-06-11T17:51:56Z

*Publisher:*

SAGE PUBLICATIONS LTD

*Published*

DOI:10.1177/0391398819860985

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## Plenary Lectures

PL111

### NEUROENGINEERING CHALLENGES IN NEUROSURGERY

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Neuroengineering is a research area where engineering skills are used to solve basic and clinical problems in neuroscience. The aim of the presentation is to give an insight into neuroengineering applied in neurosurgery.

Deep brain stimulation (DBS) is a method for reducing symptoms in movement disorders as Parkinson's disease and essential tremor. A thin electrode is surgically implanted with stereotactic technique at a well-defined brain structure. Our research focus on support systems for improving the surgical implantation procedure, and to increase the understanding of the DBS mechanism. A method for performing patient-specific simulations of the electric field around a DBS lead and map the data to the anatomy has been developed. The goal is to create improvement and side-disorder maps for the most common symptoms and brain targets used in DBS.

Optics is another core area we apply in neurosurgery. Several optical probe systems for intraoperative guidance have been developed. Forward-looking probes can act as "vessel alarm" by recording the tissue's microcirculation with laser Doppler flowmetry during creation of the brain trajectories in relation to stereotactic neurosurgery. In DBS this method has been evaluated in the clinical setting in more than 100 implantations. For brain tumor biopsies the method is presently introduced in combination with 5-ALA fluorescence measurements at the tumor border. 5-ALA induced fluorescence spectroscopy- and microscopy can also be used together in brain tumor surgery. Under the neurosurgical "blue-light" microscope, a hand-held probe supports the surgeon by enhancing malignant tissue. Another challenge is optical monitoring of cerebral microcirculation in the neuro-intensive care setting. A prerequisite for successful project outcome is a close collaboration between biomedical engineers and neurosurgeons. difference.

**Acknowledgements:** The research is supported the Foundation for Strategic Research (SSF BD15-0032), Swedish Research Council (VR 2016-03564) and the Swedish Child Cancer Foundation.

PL211

### IMAGE BASED, PATIENT SPECIFIC MEDICAL DEVICE SOLUTIONS

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Patient-specific anatomies can be reconstructed with a precision of approximately 0.5mm, using state of the art imaging modalities. This

refers both to hard and soft tissues, hence with applications in clinical domains such as orthopedics, dentistry, cardiovascular surgery. Datamining techniques such as statistical shape modeling support the generation of biofidelic computer models.

However, these models have to be fed with accurate data to reflect not only the geometry but also the material properties, the loading conditions and the interface between implants and bone in case of prosthetic reconstruction. The challenge is to optimize the interface between host tissue and implant in order to achieve maximum long-term functionality, but this optimization is also dependent on the optimization of the interfacing between the implant, the surgeon and the host tissue. Thus also the surgical technique will benefit from engineering support.

Biomedical engineering is a key player to achieve this. A good geometric fit between implant and host tissue will ensure optimal initial stability. Patient-specific implants can ensure this stability, incorporating solid and porous structures where needed. Pre-operative computer assisted planning allows to optimize the surgical intervention, incorporating patient-specific biomechanical models to predict the functional outcome of the surgical intervention. And finally, in order to achieve that the pre-operative plan is accurately transferred into the surgical practice, computer assisted surgery enabled by e.g. navigation, robotics of patient-specific surgical instrumentation (guides and implants) can come into play.

Key to all state of the art developments in computer assisted surgery is the engineering on anatomy, enabled by multifunctional softwares and 3D printing technologies. Biomechanical modeling allows for patient-specific outcome prediction after surgical interventions and should become a routine practice in patient treatment. Patient specific surgical instrumentation is able to assist in realising the plan with sub-millimeter precision. Finally, quantification of the uncertainties that are inherent to biomechanical modeling is an essential contribution to reliable patient-specific outcome prediction.

## Oral Presentations

### ECMO - From Modelling to Clinical Trial

O111

#### HYDRAULIC CHARACTERISTIC OF A MIXED FLOW BLOOD PUMP FOR AN INTRACORPOREAL MEMBRANE OXYGENATOR

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**Objectives:** A mixed flow blood pump with an outer casing diameter of 9 mm is proposed as part of an intracorporeal membrane oxygenator. The

usage of a blood pump deals with major challenges in the design of an intravenous device, specifically overcoming the pressure drop and enabling a controllable blood flow. This study presents the design, manufacturing, experimental and numerical investigation of the pump prototype.

**Methods:** The pump prototype was manufactured using a Kudo3D Titan 1 (Kudo3D Inc. Dublin, USA) 3D printer, utilizing liquid photo polymeric resin coupled with a digital light processing (DLP) curing process. A static test loop consisting of the pump, ultrasonic flow sensor and pressure sensors was used to obtain the pump characteristics at different rotational speeds ranging from 23000 to 32000 rpm. A water glycerol mixture with a dynamic viscosity of 3.4 mPa.s was selected as working fluid. Computational fluid dynamics (CFD) simulations were carried out using the open source CFD code *OpenFOAM*<sup>®</sup>.

**Results:** Over the investigated range from 30 to 500 mmHg, stable characteristic curves at different rotational speeds were measured. At 27700 rpm, a flow rate of 1.4 L/min against a pressure difference of 174 mmHg was observed. CFD simulations show a good agreement of the numerically predicted and measured pump characteristics, with an average deviation of about 8 %.

**Discussion:** The applied manufacturing process was capable to produce thin structured, complex parts down to 300  $\mu$ m wall thickness, durable enough to be used in experimental investigations up to 32000 rpm. Experimental pump characteristics show adequate performance of the proposed mixed flow pump for the intended purpose. CFD simulations accurately match the experimental pressure difference.

**Acknowledgements:** This work is supported by the Austrian Research Promotion Agency (FFG). Project: MILL – Minimal Invasive Liquid Lung. Project number: 857859.

## O112

### IN VIVO EVALUATION OF THE MODULAR EXTRACORPOREAL LUNG ASSIST SYSTEM

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**Objectives:** The Modular Extracorporeal Lung Assist System (ModELAS) is a highly compact, modular, fully integrated pump-lung. The device can be configured to provide adult low-flow extracorporeal CO<sub>2</sub> removal (ECCO<sub>2</sub>R) (removal of 30-50% of metabolic CO<sub>2</sub>), full adult respiratory support or full pediatric respiratory support. The modular design allows for the exchange of the 0.65 m<sup>2</sup> adult bundle and the 0.3 m<sup>2</sup> pediatric bundle, while all other device components are identical. These studies characterized the *in vivo* performance of the ModELAS for each application.

**Methods:** *In vivo* device performance was evaluated in healthy sheep (n=4 7-day ECCO<sub>2</sub>R, n=6 30-day adult ECMO, n=3 7-day pediatric ECMO). Pediatric studies utilized a right atrium to pulmonary artery cannulation strategy while adult studies utilized a dual lumen cannula placed in the jugular vein. Animals were recovered and tethered within a pen. The target blood flow rate was 0.5 L/min for ECCO<sub>2</sub>R and 2-3 L/min for the ECMO applications. Anticoagulation was achieved via continuous

heparin infusion. Animal hemodynamics were measured hourly, and blood chemistry, gas transfer and plasma free hemoglobin (Pfhb) were measured throughout each study.

**Results:** All animals were recovered and 9 animals completed the full study duration. Average CO<sub>2</sub> removal during ECCO<sub>2</sub>R was 74  $\pm$  6 mL/min. Average adult and pediatric oxygenation was 134  $\pm$  10 mL/min and 94  $\pm$  11 mL/min, respectively. Average Pfhb was less than 25 mg/dL, with the exception of one study following an acute kidney injury. Early terminations were due to a pulmonary embolism, a hemothorax, an intrabundle thrombus and a fractured cannula. Typical explanted devices exhibited minimal thrombus. When present, thrombi were primarily located at the bundle inlet surface and impeller pivot.

**Discussion:** These studies are on-going, but demonstrate positive performance of the ModELAS across a clinically relevant respiratory support spectrum.

## O113

### LARGE ANIMAL MODEL FOR TESTING AN ARTIFICIAL LUNG APPLIED BETWEEN THE PULMONARY ARTERY AND THE LEFT ATRIUM AS A BRIDGE DEVICE TO LUNG TRANSPLANTATION

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**Objectives:** Various modes of extracorporeal membrane oxygenation with or without a pump have been used as a bridge to lung transplantation in clinical settings. We have started to develop a large animal model for testing newly developed artificial lungs that can be used as a long-term bridging device.

**Methods:** A total of eight goats weighing 40-55 kg were used. A membrane lung (Capiiox EBS<sup>®</sup>, Terumo, Tokyo, Japan) accompanied by a centrifugal pump (Emersave<sup>®</sup>, Terumo, Tokyo, Japan) was connected to the pulmonary artery (PA) trunk for outflow, and the left atrial (LA) appendage for inflow. The PA pressure (PAP), systemic arterial pressure (AP), pre- and post-oxygenator pressures were monitored. The blood flow of the main PA and the membrane lung were monitored continuously by using an ultrasonic perivascular probe (Transonic Systems Inc., Ithaca, NY). The blood flow to the membrane lung was maintained at around 2.0 L/min during the trials.

**Results:** Four out of eight goats did not survive because of initial surgical failures (N=2), and introducing air bubbles in the membrane lungs (N=2). Other four goats were successfully kept for device testing. Durations of the circuit run were 7, 16, 24, and 48 days with or without heparin infusions according to the experimental protocols. Mean AP and mean PAP at the recovery from the surgery were 81  $\pm$  16 mmHg and 19  $\pm$  2 mmHg, respectively. The PA flow rate was 4.4  $\pm$  0.5 L/min.

**Discussion:** The animal model for testing long-term membrane lung application as PA-to-LA configuration has been established. The model makes it possible for us to assess artificial lungs *in vivo* setting for long-term with stable blood flow through the device.

**Acknowledgements:** This study was supported by the Japan Agency for Medical Research and Development (Grant number: 19hm0102 048h0003).

O114

### NO "FAILURE TO RESCUE (FTR)": SURVIVING SERIOUSLY COMPLICATED ECMO- INSERTION AS A QUALITY OF CARE PARAMETER

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**Objectives:** FTR, the death rate among patients with complications, is an emerging quality metric, that has received an increasing focus in the last years. Concerning the management and outcome in life-threatening complications due to ECMO-insertion there are only few reports available. We report the successful rescue therapy in two patients of our hospitals large ECMO-patient cohort with severe ARDS who experienced right ventricular (RV)-perforation after v-v-ECMO-cannula insertion by the Seldinger's wire.

**Methods:** RV perforation was assumed by deteriorating hemodynamics and immediate echo-cardiography showing cardiac tamponade during ECMO-cannula insertion. In cases of two patients an immediate bedside sternotomy was performed and the diagnosis verified. The source of bleeding was controlled and the tamponade was cleared. Either patient was transferred to the OR after ROSC for a definitive hemostasis and subsequent optimally controlled cannula placement.

**Results:** Both patients survived this life-threatening event without neurological complications or further bleeding as well as no infections of the wounds. In Patient 1 the ECMO-therapy was not instituted immediately but later. This patient was weaned from ECMO after one week, survived six months but finally died due to an underlying disease. In Patient 2 switching the v-v- to an a-v-ECMO in the acute setting lead to a sufficient circulation during CPR. This patient was completely weaned from ECMO after 2 weeks and was finally discharged to rehabilitation after two months.

**Discussion:** Successful management of seriously complicated ECMO-insertion is possible. The provision of an immediate bedside operative therapy and every kind of circulatory support to achieve ROSC seem to be essential in this setting. By reason of this concept a No-FTR situation could be achieved, which depicts a positive marker for quality of care for ECMO patients.

### Thrombus - Detection and Prevention

O121

### THROMBOGENICITY TESTING OF BLOOD PUMPS: IS THROMBOELASTOMETRY ABLE TO QUANTIFY THE THROMBOGENIC POTENTIAL IN VITRO?

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**Objectives:** Rotary blood pumps (RBPs) are successfully used in high-risk treatments, but clot formation still threatens their long-term application. To reduce the risk of clot formation, in-vitro thrombogenicity testing could help to improve RBP design, as several studies have shown. Those studies were able to simulate in-vitro clot formation in RBPs, but they had

limitations regarding a proper quantification of their thrombotic impact. In this study, we assessed if thromboelastometric analyses (TEM) are feasible to quantify the thrombotic impact of RBPs in-vitro.

**Methods:** Five RBPs (n = 5) were placed into simple pump circuits that were built of silicone tubes and reservoirs. Each circuit was filled with 150 ml of slightly heparinized porcine blood (one donor pig per circuit) and the pumps were brought into operation. The pumps operated until a drastic drop in volume flow indicated thrombus formation. We carefully cleaned the RBPs from blood and documented any found thrombus. Prior to this, blood samples were taken at certain time points during pump operation. The blood samples were then analyzed by TEM.

**Results:** TEM measurements showed a decrease in clotting time (CT) over the duration of the test, which indicates an ongoing increase in the activation of the coagulation system caused by the pump. Correspondingly, RBPs revealed visible blood clots at high-risk thrombus formation spots.

**Discussion:** The decrease in CT over time corresponding to clot formation in the RBPs shows that TEM is able to detect and quantify the thrombotic impact of RBPs in-vitro. Thus, TEM could be used in future studies to compare the thrombogenicity of different RBPs by performing comparison tests similar to hemolysis testing. This could lead towards a standardization of in-vitro thrombogenicity testing of RBPs.

**Acknowledgements:** This study was funded by the federal state of North Rhine-Westphalia and the European Regional Development Fund (ERDF). Grant number: EFRE-0800410.

O122

### IN VITRO INVESTIGATION OF LVAD THROMBOSIS LYSIS THERAPY

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**Objectives:** Pump thrombosis is a severe adverse event in ventricular assist devices. Current therapy often involves an exchange of the entire pump. Alternative solutions must be explored to reduce surgery numbers. A protocol for in vitro lysis of LVAD specific thrombus with alteplase was established in this study.

**Methods:** A fluid chamber of silicone tubing was filled with isotonic sodium chloride solution. An artificial human thrombus was suspended within. Alteplase was added to the system according to the maximum dose for clinical use, 0.028 mg/ml.

Five experiments each were conducted for stasis and fibrin thrombi respectively. Both types were lysis tested for four and 24 hours each. Fluid samples and photographs were taken for dissolution evaluation.

**Results:** Visual inspection of the fibrin thrombus showed clear dissolution. For the stasis thrombus, the 24 h experiment produced no discernible dissolution of the thrombi, while the thrombi investigated over the 4 h period fragmented into small pieces which did not dissolve entirely.

D-dimer levels in the fibrin thrombus tests rose steadily through the alteplase treatment for both test durations (short test 12800-25600 ng/

ml, long test 25600-51200 ng/ml). For the short term stasis thrombus testing, D-dimer values rose similarly to the fibrin thrombus, but levels remained much lower (short test 1600-3200 ng/ml, long test >200 ng/ml). Long term stasis thrombus tests showed no significant rise in D-dimer levels.

**Discussion:** Alteplase was successful in dissolving fibrin thrombi in our experimental setup. D-dimer analysis supported the visual impression. The fragmentation of stasis thrombi and D-dimer levels measured may be due to the drug's fibrinolytic effect. The amount of erythrocytes in a stasis thrombus may resist lysis and produce challenges in the clinical application of thrombus lysis treatment.

With this setup we were able to examine the reaction of the LVAD thrombi to Alteplase. This information can be used to further optimise clinical lysis therapy.

## O123

### ANTICOAGULATION QUALITY AND FREQUENCY OF INR TESTING IN LVAD PATIENTS: A CORRELATION TO HEMOCOMPATIBILITY RELATED ADVERSE EVENTS AND OUTCOMES

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**Objectives:** Anticoagulation therapy in LVAD patients is essential to reduce hemocompatibility related adverse events (HRAE). Phenprocoumon dose must be adapted and monitored by INR point-of-care-testing (POCT) in outpatients. The study aims to determine if the frequency of INR POCT in LVAD outpatients has an influence on the quality of anticoagulation therapy, HRAE and clinical outcomes.

**Methods:** This retrospective, pseudo-randomized study included n=48 patients who received an LVAD implantation (HMII, HM3 and HVAD) between Jan. 2012 and Oct. 2016. Based on the frequency of weekly INR POCT, we compared a daily (n=36) and a 3x/week (n=12) group, specifically the 1-year anticoagulation quality (% of INR Tests in Range) as well as clinical outcomes, readmissions and HRAE using Kaplan-Meier curves. Readmission profiles and outcomes in three groups, based on the achieved quality of anticoagulation (% of INR Tests in Range) ranging from 0-60% (poor), 60-70% (acceptable), 70-100% (well controlled) were compared.

**Results:** Daily and 3x/week groups were similar in demographic and pre-operative risk factors, INR target (2.0-3.0, p=0.27) and Aspirin daily doses (p=0.29). Freedom from any HRAE (38.9% vs. 25.0%, p=0.44), any readmission (72.2% vs. 75.0%, p=0.97) and 1-year survival (91.7% vs. 91.7%, p=0.98) were comparable in both groups. The % of INR Tests in Range was significantly higher with the daily self-assessments (73.5% vs. 68.4%, p=0.006). Freedom from any neurological event (91.7% vs. 75.0%, p=0.14) was n.s. higher in the daily POCT group. Well vs. poorly controlled INR POCT patients had a significant higher freedom from any neurological event (96.0 vs 69.2%, p=0.024) as well as hemorrhagic strokes (100% vs. 76.9%, p=0.011).

**Discussion:** Well controlled anticoagulation of LVAD outpatients results in less neurological events including hemorrhagic stroke. Daily INR POCT and subsequent dose adjustment of vitamin-K antagonists result in a better quality of anticoagulation than 3x/week checks.

## O124

### ANALYSIS OF LVAD LOG FILES CONSIDERING THE CIRCADIAN RHYTHM FOR EARLY THROMBOSIS DETECTION

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**Objectives:** The analysis of Left Ventricular Assist Device (LVAD) power is a commonly used method to identify complications such as pump thrombosis. However, the LVAD power is also affected by different influences like the circadian rhythm. To optimize our thrombosis detection algorithm, we analyzed LVAD log files.

**Methods:** For the investigation of power behavior in LVAD log files, we retrospectively analyzed 840 log files from 189 patients. Each patient received an HVAD (HeartWare). The log files contain on average 3000 power values collected over a period of approx. 31 days. None of the analyzed log files contained serious complications, such as a pump thrombosis.

**Results:** To show a significant difference between the power values of the daytime (8 am–10 pm) and the night-time, a statistical test (significance level: 5%) was carried out. 813 of 840 log files showed a significant difference (average t = 16.94). In addition, the average difference between the maximum and the minimum power value of a day was calculated for each day (average: 1071.9mW, SD: 158.2mW, range: 497.1mW - 2299.5mW). Furthermore, the average power was calculated for each day and log file. To get a measure for the fluctuation in daily average of power development within a log file, the SD has been calculated (SD average: 65.3mW, range 10.4mW - 435.9mW).

**Discussion:** The retrospective analysis of LVAD log files shows that power behavior is individual. Although most patients show a significant power difference between daytime and night-time hours, the maximum difference of a day and the average power changes over several days varies from patient to patient. A patient-specific power model, considering the circadian rhythm, was developed and applied to our thrombosis detection algorithms, which achieved a better thrombosis detection time of up to 93 hours compared to the LVAD controller alarming algorithm.

**Acknowledgements:** This project is funded by the German Federal Ministry of Education and Research (BMBF) within the framework of the ITEA3 Project Medolution (14003).

## O125

### INFLUENCE OF DIFFERENT ANTITHROMBOTIC REGIMENS ON PROTHROMBOTIC PLATELET FUNCTION IN PATIENTS ON MECHANICAL CIRCULATORY SUPPORT

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**Objectives:** To characterize the biological background of prothrombotic platelet function in the setting of durable Mechanical Circulatory Support (MCS) with Left Ventricular Assist Devices (LVADs) and to evaluate influence of different antithrombotic regimens dictated by specific clinical needs.

**Methods:** Platelet thrombin generation was quantified in 75 LVAD patients implanted with the HeartMate II (n=10, 13%), HeartMate 3 (n=27, 36%), or HeartWare HVAD (n=38, 51%) using the Platelet Activity State (PAS) Assay and the Thrombin Generation Test (TGT). Data was compared in patients managed with i) oral anticoagulation plus aspirin (n=46, 61%) or ii) anticoagulation alone (n=29, 39%) due to significant bleeding risk. Coagulation parameters (platelet count, International Normalized Ratio [INR], activated Partial Thromboplastin Time [aPTT], Fibrinogen and D-Dimers levels) and hemolysis index (lactate dehydrogenase levels, LDH) were also recorded to comprehensively characterize hemostatic profiles and actual prothrombotic risk in the two groups.

**Results:** The PAS assay revealed low-intensity increase in platelet prothrombinase activity in patients without aspirin (1.13-fold higher;  $p=0.04$ ). Similarly, the TGT revealed moderate higher platelet reactivity in patients not on aspirin, consistent with reduction of lag time (0.87-fold lower;  $p<0.001$ ), increase in peak of thrombin generation (1.5-fold higher;  $p=0.002$ ) and thrombin generation rate (2-fold higher;  $p=0.01$ ), but comparable endogenous thrombin potential ( $p>0.05$ ). Coagulation parameters and LDH levels were not different in these two groups ( $p>0.05$ ).

**Discussion:** In patients with a durable MCS, aspirin minimally modulates the biochemical pathway of platelet thrombin generation. Re-evaluation of antithrombotic management criteria in selected patients stratified according to bleeding risk might significantly reduce LVAD hemocompatibility-related adverse events.

**Acknowledgements:** This work was supported by Fondazione Cariplo grant #2015-1044 (awarded to FC).

## O126

### EFFECTIVE TREATMENT FOR BERLIN HEART EXCOR THROMBOSIS USING TISSUE-TYPE PLASMINOGEN ACTIVATOR THROMBOLYSIS

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**Objectives:** The Berlin Heart EXCOR is the most commonly used ventricular assist device in children. Pump thrombosis is a major complication of the system and is most likely to occur in smaller pumps used for children under the age of 2. Pump exchange is seen as the therapeutic gold standard, but is associated with high mortality if in- or outflow cannulas are affected and therefore have to be changed surgically.

**Methods:** Herein we report our experience in the use of tissue-type plasminogen activator (Alteplase, Actilyse®) for the therapy of an acute pump thrombosis subtotally occluding the inflow cannula of a 7 month old girl supported by a Berlin Heart EXCOR LVAD. The infant was supported by LVAD therapy used for acute left ventricular failure due to fulminant myocarditis, and the system was implanted 20 days prior to when the thrombotic event was reported. A treatment protocol of 1.2mg/kg - initial bolus application of 0.12mg/kg/2mins - of Alteplase infusion given continuously for 120mins was used, and the assist device was connected to a Maquet Quadrox-I neonatal and pediatric oxygenator to obviate thromboembolic complications.

**Results:** The infant supported by Berlin Heart EXCOR LVAD was haemodynamically stable prior to the thrombotic event, and anticoagulation was performed with a continuous heparin infusion. Within a couple of hours, she became tachycardic and developed signs of low cardiac output, and a subtotal occlusion of the inflow cannula of the Berlin Heart System was diagnosed. As surgery was felt to be highly problematic, we

decided to infuse Alteplase and achieved short-term success with resolution of the pump thrombosis within 2 hours. There was no adverse event.

**Discussion:** Tissue-type plasminogen activator thrombolysis might be an effective treatment for Berlin Heart EXCOR thrombosis in haemodynamically compromised children. Additionally, the use of a Maquet Quadrox-I oxygenator might minimize the risk of major complications (i.e. thromboembolism) that have been reported in the literature.

## Soft Tissue Engineering

### O131

#### NOVEL WOUND HEALING SYSTEMS BASED ON SELF-ASSEMBLED FIBRINOGEN NANOFIBERS

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**Objectives:** The protein fibrinogen is important in blood coagulation and wound healing. Hence, nanofibrous fibrinogen scaffolds are highly attractive as biomaterials. One currently used technique to prepare fibrinogen nanofibers in vitro is electrospinning. Since this technique uses organic solvents and high electric fields, which can impede the biological protein function, we have developed a novel technique to prepare fibrinogen scaffolds with physiological buffers.

**Methods:** We introduced the novel method of salt-induced self assembly under controlled drying conditions to prepare nanofibrous fibrinogen scaffolds. Scanning electron microscopy (SEM) analysis was used to characterize the fiber morphology. We studied the secondary structure with Fourier-transform infrared (FTIR) spectroscopy. To assess the biocompatibility of our novel scaffolds we analyzed the proliferation of 3T3 mouse fibroblasts with WST1 assays and studied the cell morphology with SEM.

**Results:** Salt-induced self assembly reproducibly yielded fibrinogen nanofibers with overall scaffold dimensions in the centimeter range. SEM analysis revealed fiber diameters between 200 and 300 nm. By adding a customized fixation step after the fiber formation we fabricated either immobilized or free-standing fibrinogen networks depending on the underlying substrate material. With FTIR spectroscopy we found a higher content of  $\beta$ -sheet structures for fibrinogen nanofibers in comparison to planar fibrinogen. 3T3 fibroblasts were found to proliferate well on fibrinogen nanofibers with cell numbers comparable to planar fibrinogen substrates. SEM analysis showed that 3T3 fibroblasts grew in close contact with fibrinogen nanofibers.

**Discussion:** Our new self assembly process offers a well controllable and physiological biofabrication method for large-scale fibrinogen scaffolds. Based on the good biocompatibility results our fibrinogen networks are very promising candidates for a new class of wound dressings.

### O132

#### TOWARDS THE GENERATION OF AN ATTACHABLE PRE-VASCULARIZED FIBRIN-BASED IMPLANT

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**Objectives:** Pre-vascularized, fibrin-based implants that can be attached to the blood vessels *in vivo* are a promising solution to faster and more efficient vascularization of implants, to facilitate implant healing and survival. Here, low concentrated (up to 10mg/mL) fibrin hydrogels have been shown to promote tube formation of endothelial cells in co-culture with ASC. However, these fibrin hydrogels are fairly unstable and higher fibrin concentrations are necessary for implant fabrication. We investigated whether higher concentrated (20-40 mg/mL) fibrin hydrogels were suitable for tube formation and if perfusion of the hydrogels could support capillarization of a fibrin-based implant.

**Methods:** Capillary formation of endothelial cells was tested in tube assays using fibrin concentrations of 1-30 mg/ml. Hydrogels were fabricated with cryoprecipitated fibrinogen from blood plasma that had been activated with thrombin. Gels were seeded with EC and ASC co-cultures, supplied with feeding medium containing pro-angiogenic factors, and cultivated for 7 days. Hydrogels with 40 mg/mL fibrin and pre-formed channels were cellularized with EC and ASC and perfused with feeding medium for 10 days under continuous flow.

**Results:** In tube assays, only concentrations of 1 mg/mL and 5 mg/mL fibrin were suitable for endothelial tube formation, which started after 2 days and peaked after 5 days in culture. Tubes were stable for the total assay duration of 7 days. However, under perfusion, fibrin gels of 40 mg/mL showed an endothelialized pore formation throughout the gel.

**Discussion:** High-concentration fibrin hydrogels can successfully be capillarized by EC-ASC co-cultures and perfusion. In static conditions, only low-concentration fibrin hydrogels are suitable for tube formation. As the next step hydrogels of different concentrations are to be connected to fibrin vascular grafts and perfused as a whole implant construct using a custom-made flow chamber as mechanical support.

O133

#### EFFECTS OF 2D AND 3D AMNIOTIC MESENCHYMAL STEM CELL CULTURES IN INFLUENCE TO PLATELET LYSATE

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**Objectives:** The mechano-biological behavior of mesenchymal stem cells (MSCs) in 2D and 3D cultures is determined by formation of actin filaments which occur as branched stress fibers. For clinical-scale expansion of functional MSCs, the use of xeno-based serum products is prohibited, but human platelet lysate (HPL) can potentially replace fetal bovine serum. Here we investigate actin filament formation and mitochondrial morphology in the 2D and 3D MSC culture supplemented with HPL and analyzed immunomodulatory molecules GARP, Foxp3 and IDO following pro-inflammatory stimulation.

**Methods:** Human platelet lysate composition was determined with a proteome profiler assay. Amnion derived MSCs were cultured in HPL supplemented media and MSC morphology was analyzed by flow cytometry, confocal microscopy and scanning electron microscopy. Mechano-biological properties of cultured MSCs were performed by atomic force microscopy.

**Results:** The occurrence of actin filaments in 2D MSCs cultivated in HPL supplemented media was decreased as compared to conventional growth media, where the number of ventral stress fibers, anchored at each end by focal adhesions, was affected and influenced membrane elasticity. Dorsal stress fibers or transverse arcs were not affected. Mitochondrial network dynamics in HPL cultivated MSCs showed a

perinuclear accumulation. 3D cultivation of MSCs was less sensitive to media supplementation. Functional activity of GARP was found non-stimulated MSCs while expression of Foxp3 and IDO relied on pro-inflammatory stimuli.

**Discussion:** In conclusion we can say that mechano-biological capabilities of MSCs cultivated in 2D and 3D rely on stress fibers, mitochondrial distribution and certain bioactive molecules such as GARP, Foxp3 and IDO. HPL supplementation leads to downregulation of f-actin filament formation as well as conserved membrane elasticity.

O134

#### EFFECTS OF SHORT PERIOD OF CHEMICAL MODIFICATION ON PORCINE PERICARDIUM

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**Objectives:** Autologous pericardium is one of the most ideal materials for cardiovascular reconstruction including pulmonary arteries and valvular cusps especially in pediatric patients. To improve the surgical handling, chemical pretreatments such as dehydration by ethanol (ET) or crosslinking by glutaraldehyde (GA) have been commonly attempted. In this study, we compared the effects of such chemical treatments with short period (10 min) on the mechanical properties of the porcine pericardium.

**Methods:** Porcine pericardium (n=3) was separated into 3 groups, raw group with no treatments (RAW), that under dehydration with 70% ET for 10 min (ET) and crosslinking with 0.6% GA for 10 min (GA). We measured 5 parameters of mechanical properties: the burst pressure, suture retention strength, ultimate tensile strength (UTS), ultimate strain (%) and stimulus modulus.

**Results:** Minimal burst pressure of RAW group, ET group and GA group were 12160 mmHg, 10640 mmHg, and 8360 mmHg, respectively, all of which were high enough for cardiovascular substitutes. There were no significant differences in suture retention strength (RAW:  $3.242 \pm 0.261$  N, ET:  $3.298 \pm 0.214$  N, GA:  $2.985 \pm 0.165$  N,  $p=0.529$ ), UTS (RAW:  $18.238 \pm 1.987$  MPa, ET:  $19.104 \pm 2.234$  MPa, GA:  $16.059 \pm 1.233$  MPa,  $p=0.486$ ) or Young's modulus (RAW:  $4.964 \pm 0.551$  MPa, ET:  $4.544 \pm 0.719$  MPa, GA:  $3.921 \pm 0.288$  MPa,  $p=0.408$ ). Only the ultimate strain of GA group was significantly higher between the 3 groups (RAW:  $35.64 \pm 2.82$  %, ET:  $37.99 \pm 2.38$  %, GA:  $46.39 \pm 3.96$  %,  $p=0.046$ ).

**Discussion:** Short period of chemical treatment using ET and GA might make it possible to improve the surgical handling without deteriorating the mechanical properties of pericardium.

O135

#### CELL COLONIZATION POTENTIAL OF THERMOPLASTIC SILICONE-BASED POLYURETHANE (TSPU) POLYMER FOR NOVEL HEART VALVE PROSTHESIS

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**Objectives:** To investigate the colonization potential of Ovine Endothelial Progenitor Cells (OEPs) on thermoplastic silicone-based polyurethane (TSPU) polymer under static condition.

**Methods:** Functionalised TSPU (F-TSPU) was prepared by first subjecting the TSPU nonwovens to plasma treatment, followed by sterilization with 70% ethanol for seven minutes and then coupling with VEGF under sterile conditions. TSPU without any additional treatment or modification, identified as NF-TSPU, were used as control. OEPCs were seeded at the density of  $5 \times 10^4$  cell/cm<sup>2</sup> onto square 4 cm<sup>2</sup> sized polymers under static conditions. Samples were taken at three time points: 1, 3 and 7 days. Samples were examined through live/dead staining and scanning electron microscopy (SEM) to check the progression of cellular colonization on the polymer. MTS assay was used to quantify the metabolic activity of the cells on the polymer.

**Results:** Cells seeded on the polymer surface were clearly visible on the SEM images. On day 3, the cells could be observed as patches on surface but did not cover all the surface. By day 7, the cells had almost filled the polymer surface. In case of F-TSPU, at Day 7, the underlying fiber pattern of the polymer could be observed on the cell sheet covering the polymer. Whereas this feature was not seen for NF-TSPU, indicating that the cells attached firmly to F-TSPU compared to NF-TSPU. Bright green fluorescence was observed on samples that underwent live/dead staining, indicating presence of live cells. Only a few red spots, representing dead cells, were seen. The metabolic activity on NF-TSPU was quantified to be higher than in the F-TSPU, though the difference was not statistically significant.

**Discussion:** Static cell seeding experiments showed that OEPCs were able to attach and grow on the surface of both types of TSPU. These are promising results suggesting that TSPU holds potential for engineering of a new generation of heart valve prostheses, which will be assessed in pre-clinical animal models in the near future.

O136

#### MODIFICATIONS OF MECHANICAL PROPERTIES OF IN VIVO TISSUE ENGINEERED VASCULAR TISSUES BY SHORT PERIOD OF CHEMICAL TREATMENTS

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**Objectives:** *In vivo* tissue engineered vascular tissues "Biotubes" constructed in the subcutaneous spaces of the recipients exhibited superior functions. However, since the formation of the vascular tissues depends on the recipient conditions, chemical pretreatments such as dehydration by ethanol (ET) or crosslinking by glutaraldehyde (GA) were attempted to improve their initial mechanical durability. Last year, we reported that ET and GA increased the burst pressure of the tissues and did not affect their suture retention strength. This year, we measured 3 parameters of mechanical properties: ultimate tensile strength (UTS), ultimate strain (%) and Young's modulus.

**Methods:** Tubular tissues (ID: 5 mm) constructed in the subcutaneous tissues of beagle dogs (4 weeks, n=3) were separated to the 3 groups, raw tissue group with no treatments (RAW), that under dehydration with 70% ET (ET) for 10 min and crosslinking with 0.6% GA (GA) for 10 min. We measured 3 parameters shown above, by tissue ringlet pull test.

**Results:** There were no significant differences in UTS (RAW:  $3.765 \pm 0.662$  MPa, ET:  $4.330 \pm 0.619$  MPa, GA:  $3.763 \pm 0.438$  MPa,  $p=0.409$ ) or ultimate strain (RAW:  $30.15 \pm 1.60$  %, ET:  $41.22 \pm 6.11$  %, GA:  $37.73 \pm 4.36$  %,  $p=0.209$ ) between the 3 groups. Young's modulus of ET group was significantly higher among the 3 groups (RAW:  $5.41 \pm 1.16$  MPa, ET:  $12.28 \pm 2.55$  MPa, GA:  $7.65 \pm 1.18$  MPa,  $p=0.0289$ ).

**Discussion:** Short period of chemical treatment using ET and GA might make it possible to control the mechanical properties of *in vivo* tissue

engineered vascular tissues to produce the ideal grafts in mechanical aspects.

#### VAD - Peripheral Components

O141

#### QUANTITATIVE EVALUATION OF THE FLOW AND PRESSURE IN THE PSEUDO LUMEN IN MOCK LOOP FOR THE DEVELOPMENT OF THE AORTIC STENT GRAFTS

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**Objectives:** Stent graft development is one of the most important topics for countries with a large number of elderly people. However, very little research has been carried out using model circulation of the aortic dissection. Quantitative evaluation and development have been difficult, because there was no model.

**Methods:** In this study, a fluid dynamical circuit model circulation simulating the left heart circulation of the dissecting aortic aneurysm was developed with a pulsatile blood pump. Various shapes of the inflow side and outflow side for the pseudo lumen were used in the experimental series. Furthermore, an animal model of the dissecting aneurysm was developed using a mechanical dissecting method and flow patterns of the animal aortas with the model dissecting aneurysm, after the ethical committee's approval. Flow and pressure pattern time series data was recorded in the digital recorder and a quantitative evaluation was carried out.

**Results:** Flow of the true lumen and pseudo lumen was divided with a thin membrane. Various amounts of the flow and pressure was observed and recorded. Membrane of the dissecting aortic aneurysm was simulated with rubber sheet. After the insertion of the aortic stent, the shapes of the flow and pressure pattern were altered. Animal experiments to develop the dissecting aneurysm were difficult. Firstly the aorta of the goats was removed, and then the dissecting aneurysm was to be constructed by the detachment of the tunica media of the aortic wall. Flow pattern of the experimental Aorta was recorded.

**Discussion:** Quantitative evaluation of the Aortic Stent expanding performance and pressure and flow pattern in the true and pseudo lumen had been observed in the mock circulation loop. Furthermore, animal model of the dissecting aorta will be useful material for the development of the stent graft in the near future.

O142

#### UNDERSTANDING AND MITIGATING EFFECTS OF LEFT VENTRICULAR SUCTION

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**Objectives:** Suction at the inlet of a Left Ventricular Assist Device (LVAD) is common and known to cause adverse events. It occurs when tissue is drawn towards the cannula, thus leading to a reduced or even ceased flow. Moreover, the sudden occlusion can cause flow situations inside the device to be dramatically different from typical operation. The objectives were to quantify the effect of suction on the rotor bearing of an LVAD in an advanced development status and to mitigate unfavorable effects on the ongoing therapy and pump performance.



**Methods:** A custom-made suction module was integrated into an established, active mock circulation loop upstream of an LVAD. This module comprises a membrane that can occlude the inflow cannula depending on the pressure in its fluid chamber relative to an adjustable air pressure chamber. The LVAD is equipped with rotor position sensors for online position tracking. Suction was initiated by reducing venous return and thus pump inlet pressure or by actively increasing air pressure in the respective chamber. The tests were performed with active and inactive suction speed control algorithms.

**Results:** Spontaneous suction by reducing venous return was unlikely for the LVAD under test independently of the stationary air pressure applied. By actively provoking suction, increased radial force on the rotor was observed with uncharacteristic movement of the rotor. The radial gap remained satisfactory for all operational points and patient scenarios investigated. Suction was accurately detected by the controller and the speed reduced accordingly, thus leading to a relieve of the suction event.

**Discussion:** Ventricular suction is a critical event in two ways as it negatively affects the LVAD therapy and secondly provokes uncharacteristic forces that might be critical for device performance. Future research is needed to ensure that experimental models of suction represent the variety of supported ventricles accurately.

**Acknowledgements:** The authors thank the Erich and Hanna Klessmann foundation, Germany.

O143

#### DESIGNING OF AN INDUCTIVE POWERING UNIT FOR VAD WITH PERSON SPECIFIC COIL COUPLE

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**Objectives:** The aim of this work is to study the influence of person physiology and anatomy, which include postoperative edema, movement and breathing of a patient, on the results of designing of inductive powering unit (IPU) for VAD and to propose design recommendations.

**Methods:** A characteristic feature of the IPU is the misalignment of the coils. The stability of the VAD power supply (especially with continuous powering) is very important. Therefore, it is necessary to design an IPU tolerant to coil misalignment. The procedure of geometrical optimization for increasing the stability of energy transfer of IPU was performed for 3 axial distances between coils  $d$  (8, 10, 12 mm), which is a typical misalignment with a postoperative edema. The lateral misalignment of the coils reached the value of the outer radius of the receiving coil (35 mm), which characterizes the patient movement or breathing. The operating frequency was taken 1 MHz, and the output power of the system was 10 W. The power drop was within 10%. The results were verified by numerical simulation in MATLAB and PSpice.

**Results:** It was found that with a decrease of  $d$  from 12 to 10 and 8 mm, the optimal outer radius of the transmitting coil increases from 53.9 to 54.6 and 55.7 mm. Turn pitch in the transmitting coil increases from 4.9 to 5.2 and 5.3 mm. For a receiving coil, turns pitch is increasing from 3 to 3.2 and 3.6, respectively. For all cases of  $d$  in each coil there are 11 turns (except for  $d = 8$  mm, where there are 10 turns in the receiving coil). It is recommended to design coils with a slightly larger coils turns pitch and the outer radius of the transmitting coil because of disappearance of postoperative edema.

**Discussion:** In this work, the influence of person physiology and anatomy on the design of IPU for VAD was investigated, and recommendations for designing were given.

**Acknowledgements:** This study was supported by the Ministry of Science and Higher Education of the Russian Federation (agreement No 14.579.21.0144, id RFMEFI57917X0144).

O144

#### CONTINUOUS NONINVASIVE METHOD FOR BLOOD PRESSURE MEASUREMENTS USING THE RADIAL ARTERY WITH THE HELP OF ULTRASOUND FLOW MEASUREMENT

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**Objectives:** The objective is to develop a simulator of the anatomy and hemodynamics of the radial artery in order to design a robust controller for a noninvasive, continuous blood pressure measurement method. Currently, blood pressure, in intensive care units, is measured invasively, which can lead to complications (e.g. infections). The novel approach facilitates a continuous noninvasive flow measurement, in addition to the instantaneous blood pressure measurement.

**Methods:** During measurements, a controller pneumatically adjusts the pressure in a balloon, positioned on top of the radial artery, so that the Doppler derived flow in the radial artery is reduced to a constant target value. The resulting pressure in the balloon correlates with the blood pressure. For the development of this method, a simulator, modelling the anatomical conditions of the lower arm, was constructed. The simulator was designed to create arbitrary physiological pressure curves. For the validation of the simulator, invasively measured blood pressure curves and data found in literature related to the mass flow rates were compared to the curves created by the simulator.

**Results:** The pressure curve, created with the simulator, shows a deviation of less than 5 % to the invasively measured blood pressure. The mass flow rate shows a good agreement with the literature data. The simulator is capable of simulating blood pressure curves with a mean arterial pressure of 40 mmHg to 120 mmHg. First tests with the controller have shown that it is possible to control the pulsatile flow to the constant target flow rate.

**Discussion:** Regarding the simulator, the next step is to adjust it, so that it can simulate flows above a mean pressure of 120 mmHg. Regarding the controller, stress tests must be conducted. It must be evaluated whether it is robust enough to work with pressure curves that fluctuate in mean pressure and frequency.

**Acknowledgements:** This work was supported by a grant from the Federal Ministry of Education and Research. (FKZ: 13GW0190B)

O145

#### GASTROINTESTINAL BLEEDING RATES AFTER HEARTMATE 3 IMPLANTATION

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**Objectives:** The HeartMate 3 (HM3; Abbott, USA) is a new compact LVAD which is associated with several new technical features (e.g. fully magnetically levitated pump, artificial pulse, large pump gaps, modular driveline, etc.). These benefits are supposed to lead to superior outcomes and reduced adverse events as compared to its predecessor HeartMate II (HMII; Abbott, USA) and other comparable assist devices. With this study, we investigated the effect of HM3 implantation on the occurrence of gastrointestinal (GI) bleedings compared to other LVADs.

**Methods:** We retrospectively studied a patient cohort of 595 patients who were supported with left ventricular assist devices. 170 Patients were supported by HMII, 81 Patients were supported by HM3 and 344 Patients were supported by HVAD (HeartWare, Medtronic, USA). Data

was determined through retrospective examination of medical records. Exclusion criteria were biventricular assist devices and other types of assist devices as well as LVAD exchange and re-operative procedures.

**Results:** Out of 595 patients 146 patients (24.4%) presented with gastrointestinal bleeding after LVAD implantation. In the multivariate analysis GI bleeding was significantly dependent on age but not on the BMI or gender of the patient. Statistically we were able to show that the occurrence of GI bleedings is significantly connected to general bleedings but not to cerebral hemorrhages. The subgroup analysis revealed that the occurrence of GI bleedings was higher in the HVAD (26%) and HMII (26%) groups compared to the HM3 patients (14%). However, statistical significance could not be achieved.

**Discussion:** The novel HeartMate 3 shows a promising adverse event profile with a reduced rate of gastrointestinal bleedings compared to its competitors. However statistical significance could not be achieved in this study. Thus, larger multi-center cohort analyses are needed.

O146

### IMPACT OF SPEED MODULATION SEQUENCES ON HEMODYNAMICS IN VADS: COMPARISON OF THE HEARTMATE 3 ARTIFICIAL PULSE AND HVAD LAVARE CYCLE

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**Objectives:** To investigate the impact of rotational speed modulation on VAD hemodynamics by comparing the effects induced by the artificial pulse in the HeartMate 3 (HM3, Abbott Laboratories, USA) to those induced by the Lavare cycle in the HeartWare HVAD (Medtronic Inc., USA).

**Methods:** We compared the effect of speed modulation to a baseline case with constant speed and pressure head in both HM3 and HVAD using computational fluid dynamics (CFD). CFD simulations employed high spatial (10<sup>7</sup> elements) and temporal (2° per time step) resolution and one-way coupling to a lumped parameter model (LPM) of the cardiovascular system for physiologic boundary conditions. Lagrangian particle tracking was implemented to probe viscous and total stresses along cell paths. Results were validated against experimental pressure-flow (HQ) curves.

**Results:** LPM results showed that HM3 and HVAD follow drastically different HQ loops during speed modulation, reflecting differences in pump characteristics, modulation rate and magnitude and associated fluid inertial effects. In the HM3, pump flow varied between -1.2 and 8.8 L/min during the artificial pulse vs. 3.7 to 7.1 L/min in the HVAD during the Lavare cycle. Induced aortic pressure pulses were comparable (11.3 vs 13.2 mmHg). CFD results revealed higher baseline shear stresses in the HVAD than in the HM3, coherent with previous studies. In both pumps, stresses were highest during flow acceleration. Sudden flow deceleration during the artificial pulse destabilized the flow and thereby also increased turbulence and total stresses on particles.

**Discussion:** While speed modulation may contribute to pump wash out and pulsatility, it may also increase flow disturbances and shear. By relating shear stress to the rate and magnitude of the speed changes, this study sheds light on the impact of the imposed accelerations on the stress fields. This is of relevance for the establishment of both built-in and physiologically-controlled speed modulation sequences.

### Mathematical Modelling for Hemodialysis Treatment

O151

#### A VALIDATED ANALYTICAL MODEL TO PREDICT INTERNAL FILTRATION IN DIALYZERS

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**Objectives:** Dialyzer design enhancing internal filtration may be beneficial to remove middle molecules from the patient's blood for the treatment of renal pathologies. Mathematical models have been proposed to predict the rate of internal filtration, at varying dialyzer geometry and operating conditions. However, such models generally consist of difficult differential equations, that may not be easily used and implemented in clinical practice. In this work, an analytical model to predict internal filtration, at varying dialyzer geometry and operating conditions, is presented.

**Methods:** To identify an analytical formula allowing for the prediction of internal filtration, the Poiseuille equation was used to describe axial momentum transport in the blood and dialysate compartment, assuming constant fluid viscosities, whereas radial momentum transport across the membrane was described with the Darcy equation, assuming linear ultrafiltration flux. The model was validated by comparing the pressure profiles in blood and dialysate compartments, and the rate of internal filtration predicted by the model with experimental data reported in literature for in-house TheraNova 400 dialyzer.

**Results:** An analytical formula to predict the rate of internal filtration at varying dialyzer geometry and operating conditions was obtained. Very good agreement (i.e. percentage error lower than 2%) was found between model predictions and experimental data for blood flow rate increasing from 300 to 400 ml/min, at constant dialyzer flow rate and zero net ultrafiltration flow rate. Good agreement was also found between internal filtration predicted by the analytical model presented in this work, and that predicted by using other one-dimensional mathematical models reported in literature.

**Discussion:** The analytical model presented in this work agrees well with experimental data, and can be used predict the rate of internal filtration at varying dialyzer geometry and operating conditions.

O152

#### ESTIMATION OF DONNAN FACTOR FOR MULTI-ION SOLUTIONS WITH DIFFERENT CONCENTRATIONS OF MACROMOLECULES

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**Objectives:** The Gibbs-Donnan theory describes the equilibrium of ionic solutions separated by a semipermeable membrane, when only one solution contains charged macromolecules that cannot pass the membrane. The problem was originally formulated for simple solutions, such as dissociated NaCl, which allowed to calculate the ratio between ions concentrations at the sides of the membrane (Gibbs-Donnan factor).

Solutions like plasma and interstitial fluid, and dialysate, contain however ions with different valence, and proteins in each compartment have a different concentration (and therefore charge); the classic theory needs an extension to describe these real-world scenarios. In this study we propose a new method to calculate Gibbs-Donnan factors for such more complex cases.

**Methods:** From electroneutrality considerations and the definition of Nernst potential it is possible to derive a new expression of Gibbs-Donnan factor which depends on the total concentrations of ions of the same valence. This allows, for two solutions A and B, to calculate the concentration of all ions in solution B, given: 1) macromolecule concentrations in A and B, 2) solutes concentrations in A. The transitivity of Donnan factors was proved: for 3 solutions at equilibrium (A, B, C) the factor of A to C is equal to the ratio of the factors from A to B and B to C.

**Results:** As a demonstration, the theory was applied to the calculation of the concentrations in interstitial fluid ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ ,  $\text{HPO}_4^{2-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{C}_3\text{H}_5\text{O}_3^-$ ) starting from values reported in literature. The calculated interstitial concentrations were close to the reported ones, with a global RMSE equal to 6%.

**Discussion:** The Donnan factors for small ions in biological fluids have relatively small deviation from unity, but their precise value may be of importance for ions whose concentrations are similar at both sides of the membrane, as for example sodium and calcium transport in hemodialyzers and peritoneal dialysis.

## O153

### MODEL TO DESCRIBE PROTEIN BOUND DRUG REMOVAL

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**Objectives:** Small molecule drugs can be bound to plasma proteins. Examples are Apixaban (459 Da, 87% protein bound), Carvedilol (406 Da, 98% protein bound) or Calcitriol (416 Da, 99.9 % protein bound). In plasma, these drugs have a free and a protein bound fraction. Protein binding impacts the removal behavior of the drug during dialysis. The purpose of this work was to develop a quantitative model to describe the removal of these solutes, and validate the model experimentally.

**Methods:** A mathematical model was developed by coupling the equations of transport in dialyzers reported in one of our previous studies, and mass balance equations reported in literature to include the effect of binding. Simulated HD treatments with human plasma were performed with 3 different types of commercial dialyzers having different membrane permeability to study the removal of Apixaban, Carvedilol or Calcitriol, expressed in terms of solute clearance. Additionally, the loss of albumin was quantified.

**Results:** Experimental clearance results [ml/min] are given as mean  $\pm$  MAD from 3 independent experiments in the order of Apixaban / Carvedilol / Calcitriol. Calculated values are reported in brackets. Dialyzer 1 was tested in HDF mode, yielding clearance of  $60 \pm 1.0$  [61] /  $41 \pm 1.8$  [10] /  $< 6$  [0.5]. Dialyzer 2 tested in HD mode yielded clearance of  $60 \pm 1.6$  [60] /  $31 \pm 1.6$  [10] /  $< 6$  [0.5]. Dialyzer 3 tested in HD mode yielded clearance of  $61 \pm 0.5$  [61] /  $34 \pm 3.8$  [10] /  $< 6$  [0.5].

**Discussion:** Clearance of tested drugs was lower than expected for their nominal molecular weight. The levels scaled Apixaban  $>$  Carvedilol  $>>$  Calcitriol, which is inverse to their degree of protein binding (Apixaban  $<$  Carvedilol  $<<$  Calcitriol). The degree of protein binding was revealed to be the actual critical determinant, rather than dialyzer design and operational mode. Drug clearance seemed to be driven by the proportion of the free fractions, as is reflected in the model by the increase of clearance for decreasing bound fraction.

## O154

### MONITORING OF RELATIVE BLOOD VOLUME CHANGES DURING HAEMODIALYSIS: FINDINGS FROM A MATHEMATICAL MODEL

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**Objectives:** Relative blood volume (RBV) changes during haemodialysis are typically estimated from variations in haematocrit, haemoglobin or total blood protein measured by optical, acoustic or other sensors integrated in the dialysis circuit. Due to dynamic changes in the circulation during the initial phase of dialysis (following filling of the extracorporeal circuit with the patient's blood and starting dialyzer ultrafiltration), the indications of RBV monitors depend on the exact moment of starting RBV measurements. The aim of this study was to assess this issue quantitatively using a mathematical modelling approach.

**Methods:** The developed compartmental model describes the flow of blood across the cardiovascular system and the extracorporeal circuit combined with the whole-body transport of water and the most important osmotically active solutes and the baroreflex mechanisms. The model was validated using clinical data from patients on maintenance HD. A standard 4-hour HD session with 3 L ultrafiltration was studied for a virtual reference patient with all model parameters based on the literature data. RBV changes were derived from the simulated haematocrit (HCT) changes. Two cases were analysed: the priming saline being infused to the patient or discarded.

**Results:** For the case when the priming saline was infused to the patient, the total RBV reduction at the end of the simulated dialysis session varied between 8 % (when HCT changes were monitored from the very beginning of dialysis) and 13 % (when HCT monitoring was started circa 4 minutes later). For the case when the priming saline was discarded, the analogous difference in RBV reduction was less than 1 percentage point.

**Discussion:** When the priming saline is infused to the patient, a few minute difference in the moment of starting RBV measurements may significantly affect the indicated RBV changes, which is due to the dilution of blood by the infused saline and the time it takes for the uniform mixing of blood across the circulatory system.

### Predictors of Failure in Vascular Access for Hemodialysis

## O161

### PRIMARY FAILURE OF THE ARTERIOVENOUS FISTULA IN PATIENTS WITH CHRONIC KIDNEY DISEASE STAGE 4/5

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**Objectives:** A successful creation and adequate maturation of arteriovenous fistula (AVF) provides efficient treatment and long term patient survival. The aim of the study was to determine the predictors for primary failure of AVF, such as gender, age, AVF site of creation, and primary renal disease, in patients with chronic kidney disease (CKD) stage 4/5.

**Methods:** The medical records of 178 created arteriovenous fistulae in patients with CKD stage 4/5, in a single center for the year 2018, were retrospectively studied. Primary failure of AVF was defined as thrombosis or inability for cannulation of the fistula within 3 months of creation.

Early thrombosis of AVF was defined as an immediate failure due to thrombosis of the fistula within 24 hours of creation. Adequate maturation of AVF was defined as successful cannulation of AVF for efficient HD.

**Results:** The mean age of the patients was  $59.75 \pm 14.65$  years, and 65.16% (116/178) were men. Adequate maturation of fistulae was achieved in 83.71% (149/178). Primary failure of AVF occurred in 16.29% (29/178) of the created fistulae, while 10.11% (18/178) had early thrombosis. Distal AVF (radio-cephalic) was the dominant site with 38.76% (69/178) of the created fistulae, followed by middle-arm AVF (radio-cephalic) site in 32.02% (57/178) and proximal AVF (brachio-basilic) site in 29.22% (52/178) of the created fistulae. The distal fistulae were significantly more frequently created in male patients (51 vs 18;  $p=0,015$ ). The female patients were significantly older than the male patients (63.27 vs 57.86 years;  $p=0,018$ ). The patients with diabetes mellitus (DM) were significantly older than the non-DM patients (63.65 vs 58.05 years;  $p=0,018$ ).

**Discussion:** Hospital strategy for creation of a permanent vascular access for hemodialysis is an "Arteriovenous fistula first". The female gender was more frequently associated with primary failure of AVF, while age, AVF creation site, and primary renal disease were not.

O162

### ANGIOGRAPHY AND PHLEBOGRAPHY IN A HEMODIALYSIS POPULATION. A RETROSPECTIVE ANALYSIS OF INTERVENTIONAL RESULTS.

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**Objectives:** To clarify the reasons, beneficial effects and duration of AVF patency after radiological interventions in AVF stenosis.

**Methods:** In 174 patients, 522 radiological investigations and endovascular treatments such as percutaneous trans luminal angioplasty (PTA) were analyzed, retrospectively. All investigations were performed due to clinical suspicion of impaired AVF function.

**Results:** Arterial stenosis was significantly more frequent among patients with diabetes mellitus ( $p < 0.001$ ) and interstitial nephritis ( $p < 0.001$ ). According to the venous stenosis, the diagnosis did not affect the frequency ( $p=0.22$ ), neither the degree of the stenosis ( $p=0.39$ ). The degree of stenosis prior to PTA correlated significantly with the degree of remaining stenosis after intervention ( $p<0.001$ ). Of the 174 patients, 123 (71%) performed a total of 318 PTA investigations. Repeated PTA was performed significantly more often in patients with diabetic nephropathy. The median times to first PTA and to the subsequent PTAs were 9.5 months and 5 months, respectively.

**Discussion:** Patients with diabetic nephropathy are at a greater risk of getting recurrent stenosis; this motivates a closer follow up for these patients. Clinically significant stenosis should be dilated as meticulously and as soon as possible. Occlusions of the AVF in most instances can be successfully thrombolysed or dilated upon early diagnosis.

**Acknowledgements:** Skaraborgs County, Linköping University, Njurföreningarna Norrland.

O163

### MORPHOLOGICAL AND HEMODYNAMIC CHANGES IN A PATIENT-SPECIFIC ARTERIOVENOUS FISTULA FOR HEMODIALYSIS

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**Objectives:** Native arteriovenous fistula (AVF) is the preferred vascular access for hemodialysis, but it still has high rate of failure due to stenosis formation. Convincing evidence supports a key role of local hemodynamics in vascular remodeling, suggesting that disturbed flow conditions may be related to stenosis development. The purpose of our investigation was to explore the feasibility of coupling non-contrast enhanced MRI and high-resolution computational fluid dynamics (HR-CFD) to relate morphological vessel changes to local hemodynamics in AVF over time.

**Methods:** We acquired non-contrast enhanced 3D fast spin echo MRI (CUBE T1) at 1 and 6 weeks, 6 months and 1 year after radio-cephalic AVF creation in one patient. We generated 3D models and evaluated lumen cross-sectional area changes over time. We performed CFD simulations using pimpleFoam solver of OpenFoam, prescribing blood flow waveforms derived by Ultrasound examination. We computed the 2 components of the wall shear stress vector over time, namely WSSdir, the component in the mean direction of the WSS vector and WSStr, the transversal component.

**Results:** We observed a dilatation of the vein until 6 months, with a more pronounced increase in the venous outflow as compared to the juxta-anastomotic vein (JAV). The increase in vein's diameter was then followed by a narrowing of JAV at 1 year after AVF surgery. We found high-frequency fluctuations both for WSSdir and WSStr components, in different locations of the vein, at 6 weeks and 6 months after AVF creation. Oscillations of both components damped at 1 year after AVF creation, as a result of vessel remodeling.

**Discussion:** Optimized CUBE T1, coupled with HR-CFD, allowed a characterization of morphological and hemodynamic changes over time. Our MRI-to-CFD pipeline represents a promising approach to elucidate mechanisms of local vascular remodeling and can be used for clinical investigations aimed at identifying critical hemodynamic factors responsible for AVF failure.

O164

### EXPLORING THE POTENTIAL OF SOUND ANALYSIS TO DETECT STENOSIS IN ARTERIOVENOUS FISTULAE FOR HEMODIALYSIS

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**Objectives:** Native arteriovenous fistula (AVF) is the preferred vascular access for hemodialysis (HD), but it still fails in more than 40% cases within the first year. Stenosis is the main cause of AVF failure, but its detection and prediction are still open clinical challenges. The purpose

of our study was to explore the potential of using sound analysis to reveal unique characteristics in AVF sounds and to detect potentially relevant changes over time.

**Methods:** We acquired the sounds of 10 AVFs, 7 referred by the nephrologists as well-functioning and 3 as stenotic AVFs. We also acquired the sound of 1 AVF characterized by severe stenosis and we repeated the recording after surgical revision. Sounds were recorded using the Littmann Electronic Stethoscope 3200 using wide mode. Sounds were transmitted via Bluetooth to a laptop and frequency spectra were obtained using an in-house Matlab code embedding the Fast Fourier Transform and a high-pass filter with a cutoff frequency of 50 Hz, aimed at attenuating stethoscope's noise and heart sounds' frequencies.

**Results:** In well-functioning AVFs we consistently found a low-frequency peak, located in the bandwidth 100-200 Hz, while no relevant spectral features were present at higher frequencies. On the other hand, stenotic AVFs showed high-frequency peaks, located in the bandwidth of 500-600 Hz. In the AVF with severe stenosis we found high-frequency peaks in the bandwidth 700-750 Hz, which were replaced by low-frequency peaks after AVF revision.

**Discussion:** Sound analysis revealed unique characteristics in the frequency spectra of AVFs, allowing an objective discrimination between well-functioning and stenotic AVFs. This technique may bring the advantage of limiting clinician's skill-dependency and personal interpretation. Despite being preliminary, our results suggest that sound analysis may be used to detect stenosis development and allow patients' self-monitoring, resulting in early prediction of AVF failure.

O165

#### THE ROLE OF CALCIUM AND PHOSPHATE IN MEDIAL VASCULAR CALCIFICATION

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**Objectives:** Vascular calcification is accelerated and worsened in chronic kidney disease (CKD) and dialysis patients compared to the general population. Misbalanced phosphate and calcium metabolism plays an important role in this health burden in CKD. In this study the role of phosphate and calcium in inducing medial vascular calcification is researched.

**Methods:** Rat aortic rings were incubated in the normal and calcifying mediums for seven days. Three independent experiments, three rings per condition in each, were performed (N=9).

Normal medium consisted of DMEM with 0.9 mM phosphate and 1.8 mM calcium. Calcium medium (Ca) consisted of 4.0 mM calcium and 0.9 mM phosphate; phosphate medium (P) of 2.8 mM phosphate and 1.8 mM calcium; and calciumphosphate medium (Ca+P) of 4.0 mM calcium and 2.8 mM phosphate.

Histochemical von Kossa staining was performed, and calcification area of a medial and adventitial section of the aorta was calculated. The results were compared using a two-way ANOVA test.

**Results:** Von Kossa stainings and evaluations of the calcified area demonstrated increased calcification in aortic rings incubated in different calcification mediums, whereas the calcification takes part in different sections of the aorta. Calcium alone causes calcification in the adventitia, phosphate alone in the medial section of the aorta. Combination of both leads to extensive calcification in the adventitia and media.

**Discussion:** In dialysis patients, serum calcium levels are maintained quite well, but serum phosphate levels may exceed suggested levels

multiple times. The results of the study demonstrate that high phosphate level is the trigger of excessive vascular calcification and the control of levels of phosphate in CKD patients is crucially important.

**Acknowledgements:** The research was funded partly by the Estonian Research Council grant PUTJD66 and IUT 19-2 (to J.H.). Furthermore, the study was supported by a grant from the German Research Foundation (DFG, SFB/TRR219 project C-04 to J.J.).

O166

#### ANALYSIS OF TRENDS IN UREA AND CREATININE CLEARANCES DURING HEMODIALYSIS SESSIONS

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**Objectives:** It has been observed that the in-vivo dialyser clearance for small solutes is not constant during a hemodialysis (HD) session. This may influence the choice of the value to be measured and reported. We investigated the presence of trends in the clearances of urea and creatinine, and how it impacts their values.

**Methods:** Diffusive clearances were calculated for urea and creatinine from dialysate concentration data measured in 25 patients who underwent 3 consecutive HD sessions each. Clearance values were obtained for each hour (at 0, 60, 120, 180, 240 minutes). The significance of the observed trend in the data was statistically tested, as was any difference between the values at 2 and 3 hours, and the average clearance during the session.

**Results:** Urea clearance showed an overall increase from the start of HD (by  $+10.1 \pm 19.3$  mL/min,  $p = 0.0002$ ) while creatinine clearance decreased (by  $-30.7 \pm 17.1$  mL/min,  $p < 10^{-4}$ ). For urea, there was, however, a significant increase during the first hour (by  $+26.5 \pm 16.3$  mL/min,  $p < 10^{-4}$ ) which was not observed for creatinine. Starting from 2 hours, both clearances decreased significantly, but the overall drop for creatinine was twice that for urea (by  $-34.1 \pm 15.7$  vs.  $-16.3 \pm 19.8$  mL/min,  $p < 10^{-4}$ ). As a consequence of these different trends, clearance at 2 h, 3 h, and the time-average clearance were not significantly different for urea ( $199.2 \pm 25.9$  mL/min,  $p = 0.18$ ), whereas they were so for creatinine (2 h:  $115.9 \pm 26.3$  mL/min vs. 3 h:  $106.2 \pm 29.2$  mL/min vs. average:  $109.7 \pm 26.1$  mL/min,  $p < 10^{-4}$ ).

**Discussion:** A decrease in clearance during HD can be expected because of the progressive clogging of the dialyzer membrane's pores. It is not clear however the reason for the higher decrease in creatinine compared to urea. Our results suggest anyway that the time at which creatinine clearance is measured in-vivo should be clearly reported, as the difference during the session might be as high as 30%.

#### Smart Tissue Freezing and Quality Control

O171

#### CRYOPRESERVATION IN TISSUE MEDICINE: THEORY AND PRACTICE

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**Objectives:** Cryopreservation plays a major role in the storage of tissues that are kept for subsequent transplantation. A wide variety of tissues

intended for transplantation for a patient are stored in this way. In addition to the assignment to a suitable recipient, it must be ensured in particular that a sufficiently long period of time is available for infection diagnostic analyses. Cryopreservation of tissue is therefore routine in everyday tissue medicine.

**Methods:** As is well known, the success of low temperature storage depends in particular on coordinated freezing and storage processes, in the optimization of which many research approaches have already been investigated. In contrast to these well-known principles of important parameter adjustment in this context, little attention is paid to optimizing the freezing process in the daily routine of tissue medicine. In fact, for some applications the suboptimal storage of tissue is not critical, as no vital cells are necessary or desired.

**Results:** This reduces the process to a minimum and eliminates the need for additives such as cryoprotective agent (CPA) or animal/human serum. This is particularly important for tissue preparations which, as in Germany, require a medicinal product approval by a competent authority. Any change to the process must be first approved by the competent authority, the Paul-Ehrlich-Institut (PEI). Optimizing the freezing process would therefore entail a new approval procedure. If the changed process was not previously known in the EU, a clinical study is also required.

**Discussion:** For this reason, the conditions are not improved in case of doubt, although it is known that this could lead to a higher quality of the tissue after thawing. Less toxic CPA could simplify the approval procedure and thus contribute to improved tissue storage for transplantation. The same applies to the cryopreservation of tissue engineered products.

O172

### CRYOPRESERVATION DOES NOT AFFECT THE REGENERATIVE POTENTIAL OF HUMAN AMNIOTIC MEMBRANE

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**Objectives:** Amniotic membrane (AM) is applied in variety of clinical settings, mainly in wound healing and as a biological dressing. Currently, AM is used in ophthalmology for the treatment of cornea pathologies. In this study, the regenerative potential of human amniotic membrane after cryopreservation was evaluated.

**Methods:** AM was prepared under sterile conditions and frozen without cryoprotectants at -80°C. Native non-cryopreserved AM served as a control. The strategy for AM cryopreservation included one or two freezing and thawing cycles. Cytokine and growth factor profiles as well as morphology and mechanical properties of AM were compared before and after one and two cycles of cryopreservation. Levels of EGF, HGF, TGF- $\beta$ 1, bFGF, Laminin and Hyaluronic acid were measured by ELISA. Surface structure of the membrane was analyzed by scanning electron microscopy (SEM). Morphology was evaluated by histological analyses after Hematoxylin Eosin (HE) staining. Additionally, biomechanical characteristics, such as the modulus of elasticity (Young's modulus) and the tensile strength, were also investigated.

**Results:** EGF, HGF, TGF- $\beta$ 1, bFGF, Laminin and Hyaluronic acid were detected in all studied samples. No significant differences were detected in the levels of those cytokines before and after cryopreservation. SEM

analysis showed minor structural alterations of double cryopreserved samples. Analysis of HE stained AM showed minor changes between native and frozen membranes, such as few areas with desquamated epithelium which was caused by repeating freezing and thawing cycles. Mechanical tests did not reveal relevant changes between frozen and non-frozen samples.

**Discussion:** Multiple cryopreservation steps are an essential part of manufacturing of human amniotic membranes to ensure a ready-to-use product availability. This study demonstrated that the cryopreservation does not impair the regenerative potential of AM required to support its therapeutic efficacy.

O173

### LOW TEMPERATURE STORAGE OF NATURAL AND BIOENGINEERED MULTICELLULAR PLACENTAL CONSTRUCTS

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**Objectives:** Placental complex, including placental tissues, foetal membranes, and umbilical cord, is one of the most promising sources of a range of tissues and cells for clinical and experimental application in regenerative medicine and tissue engineering. Certain components of placental material are already applied in medicine since decades, while others undergo intensive research and pre-clinical trials. Since placenta is a temporary organ, it provides the unique opportunity to obtain large amounts of tissues, including autologous, without the adverse effects to the donor. Moreover, great potential of placental multipotent stromal cells is recognized in generation of tissue engineered multicellular constructs. At the same time, clinical use and research proceedings are significantly restricted due to imperfections in storage technologies. Application of low-temperature preservation methods is especially challenging for 3-D multicellular structures, such as organs, tissues, and tissue engineered constructs.

**Methods:** Here we aim to analyze the variety of cryoinjuries in a range of placental tissues and tissue engineered multicellular placental constructs, as well as support the cellular viability after low temperature storage. A range of cell and tissue culture as well as tissue engineering methods, cryomicroscopic analysis, evaluation of phenotypic and metabolic characteristics have been applied in this study.

**Results:** Results have shown that the mechanisms of damage caused by cryopreservation procedures depend on particular structure of the studied object, peculiarities of intercellular bonds, as well as interaction with cryoprotective agents. At the same time, developed protocols allowed us to efficiently isolate viable cells after thawing of the studied tissues.

**Discussion:** Conclusions of this study may lead to progress in improvement of biobanking technologies for tissues and tissue engineered multicellular constructs.

O174

### FROSTY MATTERS: DO FREEZING CONTAINERS AND SMARTFREEZERS MAKE THE GRADE?

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**Objectives:** The optimization of the long term storage of cells and tissues is a challenging process with many variables but one factor is often overlooked: the freezing device itself. There are freezing containers that have to be placed in a -80°C freezer and the manufacturer promises a cooling rate of 1 K/min. On the other hand there are controlled rate freezers where cooling rates from 0.1 K/min up to 50 K/min are promised. In this study we compared two commercially available freezing containers and four controlled rate freezers with respect to their functioning principle. Furthermore, we investigated the accuracy of the adjusted cooling rate and the nucleation temperature of the samples.

**Methods:** Seven 1.5 ml cryovials filled with 1 ml 0.9% (w/v) sodium chloride solution were dispersed evenly over the rack of each freezing device. Constantan thermocouples (type T) connected to a RedLab device were placed in the middle of the solution of each cryovial to record temperatures every second with the respective software. The cooling rates were calculated from the melting point (~-0.6°C) of the solution to -30°C with n=3. For all freezing devices a cooling rate of 1 K/min was set according to the manufacturers guidelines.

**Results:** The freezing containers had sample cooling rates between 0.5 and 0.8 K/min. All controlled rate freezers showed cooling rates similar to the programmed cooling rate of 1 K/min. Higher cooling rates resulted in increasing deviations between programmed and measured cooling rates. The nucleation temperatures of the samples in the freezing containers were mainly between 0°C and -6°C. However, the controlled rate freezers showed nucleation temperatures mainly in the range of -6°C to -12°C.

**Discussion:** Varying definitions from each manufacturer resulted in the observed cooling rate differences of the freezing containers. Limited heat transfer accounted for the increased cooling rate deviation recorded in higher programmed cooling rates.

**Acknowledgements:** This work was partly supported by the German Research Foundation, through the Cluster of Excellence REBIRTH EXC 62/1. The Institute for Multiphase Processes was/is collaborating with Asymptote and Askion in projects that are not part of this study.

O175

### QUANTITATIVE PHASE IMAGING FOR CELLULAR PROCESS CHARACTERIZATION AND CELL QUALITY CONTROL

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**Objectives:** Quantitative phase microscopy (QPM) provides fast and label-free analysis of living cells by minimized interaction with the sample. The analysis of QPM images enables the determination of morphological cell parameters like cell thickness and elongation and facilitates single cell tracking for migration analysis.

**Methods:** To demonstrate the potential of QPM in determining the metastatic potential of cells, we have developed a fully automated QPM system utilizing digital holographic microscopy (DHM) in an off-axis Mach-Zehnder configuration. Different pancreatic tumor cell lines, beating heart cells, white blood cells and in vitro toxicology models were analyzed for refraction index, cell volume, cell area, dry mass and cell morphology dependent processes.

**Results:** The morphological analysis of the different cell lines via QPM in combination with image segmentation-based evaluation of the retrieved quantitative phase images showed that QPI could identify and quantify cell types and cellular processes by cell specific properties as cell proliferation, cell death, 3D cell migration or cell height. E. g. cells with a high metastatic potential had a lower cell thickness and a higher elongation than cells with a low metastatic potential. Moreover, computer assisted

tracking of single cells showed that highly metastatic cells covered a longer distance and had a higher motility compared to cells with a low metastatic potential. Also white blood cell types and disease or drug induced morphological blood cell alterations could be separated.

**Discussion:** In summary, the multi-functional potential of QPM in various cell biological applications as cancer cell or stem cell research, toxicological and pharmacological screenings and cell quality control represents a promising new approach for a fast and label free phenotyping of different cell types and cellular processes. QPM meets the growing interest in label-free, optical techniques minimizing samples interaction.

O176

### CRYOPRESERVATION OF CELL-SEEDED ELECTROSPUN MATERIALS: TOWARDS BIOBANKING OF TISSUE-ENGINEERED CONSTRUCTS

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**Objectives:** Cryopreservation of tissue-engineered constructs (TECs) is very important to provide such ready-to-use products for regenerative medicine and clinical application upon demand. Although cryopreservation of isolated cells seems to be well established, there are still a number of challenges associated with the cryopreservation of native and artificial tissues due to adherent cell state, limited heat and mass transfer as well as inadequate cryopreservation protocols. Here, we aim at developing an approach for efficient cryopreservation of electrospun TECs based on multipotent stromal cells (MSCs).

**Methods:** Blend electrospun fibre mats (fibre diameter  $0.8 \pm 0.2 \mu\text{m}$ , thickness  $100 \pm 10 \mu\text{m}$ ) were produced from polycaprolactone and polylactic acid (PCL-PLA, ratio 100:50) using electrospinning. The fibre mats (diameter 16 mm) were UV sterilised and seeded with MSCs ( $5 \times 10^4$  cells/cm<sup>2</sup>). The cells were cultivated on fibre mats for 7 days under static conditions and then frozen using 1 K/min cooling rate in a controlled rate freezer with different formulations of cryoprotective agents (CPAs), such as dimethyl sulfoxide (DMSO) and its combination with sucrose (with and without pre-culture with sucrose for 24 h). The viability of cells growing on fibre mats was monitored for 2 weeks after seeding and 24 h after thawing.

**Results:** The results indicate that PCL-PLA fibre mats are biocompatible with MSCs (viability higher than 82%). Pre-culture with sucrose before freezing as well as its addition to DMSO-containing freezing medium significantly improved cell viability after thawing. Moreover, duration of equilibration of cell-seeded fibre mats with the CPAs before freezing affected cell viability post-thaw.

**Discussion:** We showed that it is feasible to effectively cryopreserve electrospun TECs using controlled technological steps. This work could serve as a solid background for further development of efficient cryopreservation methods for biobanking of electrospun constructs for vascular or corneal tissue engineering.

### Measurement Methods in the Circulatory System

O211

### ECHOCARDIOGRAPHIC FLOW FIELD VISUALIZATION DURING MECHANICAL CIRCULATORY SUPPORT IN THE ISOLATED ASSISTED HEART

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**Objectives:** Intraventricular flow patterns during mechanical circulatory support (MCS) cannot be accessed by clinical imaging; therefore, either computational or in-vitro models are used. However, the complex anatomy of the heart cannot be replicated and simulations inherently rely on assumptions and simplifications. In an isolated porcine heart setup the feasibility of flow measurements by Echocardiographic Particle Image Velocimetry (E-PIV) was evaluated.

**Methods:** Similar to cardiac transplantation, porcine hearts (n=8, animal weight: 80-106 kg) were excised and connected to the isolated heart setup. After resuscitation using blood as perfusate, a rotary blood pump was implanted, microbubbles were injected via the left atrium at different support situations and echocardiographic 3-chamber-view B-mode images were recorded with the highest possible frame rate of up to 141 Hz (Philips iE33, X5-1 xMatrix probe). By iterative PIV algorithms using correlation domain averaging and beam sweep correction, flow fields were evaluated for the different hemodynamic situations.

**Results:** All hearts were successfully resuscitated in the isolated heart setup and different hemodynamic situations were adjusted. In the unsupported heart physiologic flow patterns with a large clockwise vortex structure that warrants washout of the whole cardiac chamber were found. With increasing MCS (2200-2700 rpm) the formation of this flow feature is diminished caused by the additional flow sink at the apex. In full support, without aortic valve opening in the left ventricular outflow tract, a stagnant structure was identified, that might be connected to thromboembolic events.

**Discussion:** For the first time, the contribution of the mitral valve apparatus to blood flow patterns especially in the LVOT, which may be linked to energy loss, thrombus formation and valve deterioration during MCS was investigated under realistic conditions.

## O212

### BLOOD FLOW RATE ESTIMATION METHOD FOR AN INTRACORPOREAL MEMBRANE OXYGENATOR

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**Objectives:** The blood flow rate is the only parameter that can be controlled during the operation of an intracorporeal membrane oxygenator that significantly influences the gas exchange rate. Due to anatomical size restrictions, the catheter is of limited size and the tight packaging does not allow for placement of a dedicated flow rate sensor. Therefore, for a flow rate control system, the correct estimation of the blood flow rate is essential. In this work, a system specific flow rate estimation method is presented based on motor current and motor speed measurements.

**Methods:** A training data set of motor current -  $I$  and rotational speed -  $\omega$ , as well as volumetric flow rate -  $Q$  were recorded in an in-vitro model

of the intracorporeal membrane oxygenator. A 40/60 water/glycerol mixture was used to approximate the dynamic properties of blood. The rotational speed of the motor was varied in a range of 10000 to 30000 RPM. For each motor speed, the hydraulic resistance of the system was varied with a throttle resulting in different flow rate values. Additionally, a test data set was acquired consisting of different rotational speed, hydraulic resistance, and flow rate triplets. The  $I$ ,  $\omega$  and  $Q$  training data was used to construct a fitting surface of the type  $Q(I, \omega)$  by a regularized cubic spline least-squares approximation method. The surface was defined in a grid of 591x591 points with  $I_{min}=-70$  mA,  $I_{max}=520$  mA,  $\omega_{min}=500$  RPM,  $\omega_{max}=30000$  RPM. The test set of  $I - \omega$  pairs was then used to predict  $Q$  by means of a nearest neighbor search in the modeled surface.

**Results:** A correlation coefficient of  $r=0.96$  was achieved between the estimated and measured flow rates of the water/glycerol mixture.

**Discussion:** The strong correlation between estimated and measured flow rate suggests that sensorless flow rate control is possible.

**Acknowledgements:** This work is supported by the Austrian Research Promotion Agency (FFG). Project: MILL – Minimal Invasive Liquid Lung. Project number: 857859.

## O213

### INTERVENTRICULAR DYSSYNCHRONY DURING CONTINUOUS-FLOW LEFT VENTRICULAR ASSIST DEVICE SUPPORT

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**Objectives:** We focused on the interventricular dyssynchrony caused by the shortened systole of left ventricle (LV) compared to right ventricle (RV) during left ventricular assist device (LVAD) support. The purpose of this study was to assess and quantify the mechanical interventricular dyssynchrony according to the support condition using conductance catheter.

**Methods:** We studied five goats with normal heart condition. A centrifugal LVAD was implanted under general anesthesia. We inserted the conductance catheter into the LV and RV and obtained the pressure-volume relationship. We defined the dyssynchronous status by the sign (plus or minus) of the LV volume (LVV) change is opposite to that of RV volume (RVV). (i.e.,  $(dLVV/dt) \cdot (dRVV/dt) < 0$ ). Interventricular dyssynchrony (DYS) was quantified by calculating the percentage of time within the cardiac cycle. Bypass rate was set dividing the LVAD pump flow by the main pulmonary artery flow. We calculated the DYS under LVAD support with various bypass rate.

**Results:** The mean DYS of normal heart, LVAD clamp, LVAD support with bypass rate of 50%, 75 % and 100% were  $6.0 \pm 1.3$ ,  $8.6 \pm 2.6$ ,  $9.6 \pm 1.8$ ,  $15.5 \pm 5.6$ , and  $24.6 \pm 8.6$ , respectively. Also, there was a higher linear negative correlation between LV stroke volume and DYS.

**Discussion:** Interventricular dyssynchrony during LVAD support was assessed and quantified using conductance catheters. Dyssynchrony became significant when the LV was unloaded with high rotational speed under LVAD support. Assessment of influences for the right ventricular function and heart failure models will be studied in the further study.



O214

### DEVELOPMENT OF BLOOD VESSEL MODEL WITH PRESSURE DISTRIBUTION SENSOR FOR IN VITRO EVALUATION OF ENDOVASCULAR DEVICES

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**Objectives:** In developing endovascular treatment devices, understanding their physical properties is one of the most important points. We have been developed film-type sensor for force measurement, applied to the field of biomechanics. In this study, the blood vessel model embedded pressure film sensor was fabricated and the contact pressure distribution in the circumferential direction was measured at balloon dilatation.

**Methods:** The film sensor was constructed by a couple of patterned electrodes and pressure-sensitive layer. The film sensor was rolled and embedded to straight-shape silicone vessel model. The balloon catheter was expanded in a mock vessel.

**Results:** The measured pressure at the mock vessel increased according to balloon dilatation pressure. At expanding of general balloon, the measured pressure at each point was almost the same. When expanding a scoring balloon, a balloon with wedge elements, the measured pressure at wedge contact points was higher than that of the other parts.

**Discussion:** It is considerable that stress concentration state changes depending on the wedge shape and/or stiffness. The mock vessel is useful to understand dilatation property of scoring balloon.

**Acknowledgements:** This study was supported in part by JSPS KAKENHI Grants in Aid for Scientific Research (No. 18K18352).

O215

### LAP ESTIMATION BASED ON HEART PUMP CHARACTERISTICS USING MACHINE LEARNING APPROACH

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**Objectives:** In patients with circulatory support, preload is a valuable clinical monitoring variable. While it is difficult to measure directly, it can be indirectly measured by PCWP or left atrial pressure (LAP). Typically, assessments of LAP require invasive methods. However, non-invasive estimation of LAP can be a useful tool for monitoring patients and adjusting pump speed to reduce the likelihood of hazardous events, such as ventricular suction and pulmonary congestion. This study presents an artificial neural network model estimating the LAP noninvasively across different patient conditions.

**Methods:** LAP was estimated based on the HeartWare HVAD pump characteristics (current, flow and speed) derived from 186 experiments performed in a mock circulatory system with HVAD speed range from 1800 to 3600 rpm. The LAP estimation was evaluated by measuring the hemodynamic variables in response to a range of changes to the mock cardiovascular system, including changes in PVR, SVR and HR. A neural network was trained with 70% of the data (randomly chosen), and was validated and tested with the rest of data. The model performance was quantified by the mean squared error (MSE), correlation coefficient ( $r^2$ ) and Bland-Altman plot between measured and estimated LAP in different scenarios.

**Results:** The neural network model was able to estimate LAP accurately using data collected in-vitro for training, validation and test sets. The

MSE for all data is 1.36 mmHg, and correlation coefficients are 0.99, 0.91 and 0.94 for training, validation and test sets, respectively. The Bland-Altman plot showed that error has a bias of 0.28 mmHg with a reproducibility coefficient of 2.2 mmHg.

**Discussion:** The developed model would be useful for a real-time estimation of preload noninvasively which can be employed to adjust pump speed in heart-failure patients without inserting a pulmonary catheter into the pulmonary artery. Implementation of the developed estimator would also create a new physiological control system that can work noninvasively.

O216

### TRANSPARENT TWO-PHASE BLOOD MODEL FLUID FOR UPSCALED MODELS

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**Objectives:** The objective of this work is the development of a two-phase blood model fluid for the use in upscaled flow models. In large scaled models of different flow geometries, frequently used water-glycerol mixtures are not sufficient because in critical regions with small gaps, e.g. between the rotor and the wall in cardiac support systems, the two-phases of blood have a great influence on the flow. Therefore, it is necessary that the model fluid represents both phases of blood: the plasma and the cells.

**Methods:** Transparent alginate beads were used as a model of red blood cells. A water-glycerol mixture was used as a model fluid for plasma. The alginate beads made up about 40 % of the volume to model the proper hematocrit. Furthermore, the beads have a slightly higher density than the surrounding medium and keep floating once in motion. This was achieved by embedding hollow glass particles in the spheres that have a density <1.0 kg/l, also enabling particle tracking and flow analysis using the Particle Image Velocimetry method. A camera tracks the flow path of the beads through the required geometry. Reynolds similarity was maintained.

**Results:** Flow analysis has been performed in an upscaled model of blade and gap of a ventricular assist device. Compared to a water-glycerol mixture, the proposed blood model fluid enabled the visualization of the strong influence of the two phases on the flow. Because of their transparency, it was possible to visualize the flow field at several layers in the fluid by choosing the appropriate light sections.

**Discussion:** The proposed blood model fluid showed promising results in an initial case. Moreover, the results of the experiment lead to a possible optimization of the geometry of future rotor blades in blood pumps in order to reduce hemolysis. Further channel geometries will be explored. In the long term, the model can be used for experiments on flow analysis and optimization in blood pumps or as a validation model for CFD-codes to calculate multiphase flows.

### Hydrogels and Scaffold Engineering

O222

### 3D CELL CULTURE CONDITIONS MEET PHYSIOLOGICAL HYPOXIA: ESSENTIAL PARAMETERS FOR CLINICALLY RELEVANT IN VITRO MODELS

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**Objectives:** Three-dimensional (3D) in vitro cultivation systems have gained increased attention, since using these systems allows the precise study of mammalian cell physiology, intercellular interaction, as well as cell-matrix interactions. Another important cell culture parameter, often neglected in traditional cultivation, is the concentration of dissolved oxygen. Generally, cell culture is performed under ambient oxygen saturation (21%), while the oxygen amount available in vivo in human tissue is much lower (1% to 15%). The stiffness of in vivo tissues and in vitro constructs also plays a crucial role in cell fate and physiology.

**Methods:** Semi-synthetic hydrogels with tunable properties allow creating a wide range of in vitro material stiffness. The development of hydrogel-based 3D cell culture gradient systems enables to obtain complex, but precisely defined in vitro microenvironments, which in turn allow a systematic investigation of the influence of microenvironmental conditions. Oxygen gradients in 3D and 2D cell culture can be created with the help of incubation chambers.

**Results:** Encapsulation of mesenchymal stem cells containing fluorescent hypoxia sensors reveals specific conditions where the hypoxic response is activated and determines the influence of hydrogel stiffness on the hypoxic response. In the presented study, exact correlations between in situ O<sub>2</sub> concentrations in 3D cell cultures (hydrogels and microtissues) and hypoxia inducible factor (HIF) activation are disclosed for the first time.

**Discussion:** Creating stable in vitro oxygen gradients in 3D hydrogel-based cultures with stiffness variety helps to better understand and predict cell behavior (survival, cytokine expression, spontaneous differentiation of stem cells or migration capacity) in physiological and pathological in vivo conditions, as well as to find optimal conditions to simulate the in vivo cell niche.

## O223

### THE DECELLULARIZED CHORION MEMBRANE AS A SUBSTRATE TO MIMIC BIOLOGICAL BARRIERS

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**Objectives:** The aim of this work is to characterize the decellularized human chorion membrane (dHCM) and to study its suitability as a substrate to model biological barriers.

**Methods:** HCMs were submitted to a chemical and physical decellularization process. The decellularization protocol was optimized by quantifying and analyzing the presence and distribution of cell nuclei and dsDNA by DAPI staining, PicoGreen and electrophoresis. The dHCM was characterized by scanning electron microscopy (SEM), immunohistochemistry, Dot-Blot, SDS-PAGE, and GAGs and Collagen quantification. Moreover, dHCM's mechanical properties were assessed and biocompatibility tests (metabolic and proliferation assays) with an endothelial human cell line (EA.hy926) were performed.

**Results:** The decellularization process of the HCM was successful, since the presence of nuclei was not observed, the amount of dsDNA was around 10 ng/mg of dry tissue, and the DNA fragments had less than 200 bp. A compact membrane that preserved the HCM's reticular layer and basement membrane was obtained. SEM images revealed that dHCM is

a substrate with two different surfaces: one composed by nanofibers and the other (in trophoblast layer's side) covered by a thin compact layer (basement membrane). In tissue sections it was possible to verify the presence of collagen type I and IV, fibronectin and laminin in both dHCM and native tissue. Corroborating these results higher molecular weight species were preserved in dHCM (SDS-PAGE). Additionally, dHCM's mechanical properties were determined: young's modulus of ~5.9 MPa and ultimate tensile strength of ~5.3 MPa. Differences in metabolic activity and cell proliferation were observed between both sides of the dHCM.

**Discussion:** dHCM retained the main proteins of the extracellular matrix. Cell proliferation and viability were higher in the side of dHCM that preserved the basement membrane. dHCM's physical and mechanical properties make it a suitable substrate to mimic biological barriers.

**Acknowledgements:** FCT-PhD grant, FRONThERA-RL3.

## O224

### MULTIPOTENT STROMAL CELL GROWTH ASSESSMENT ON POLYCAPROLACTONE-GELATIN HYBRID ELECTROSPUN SCAFFOLDS

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**Objectives:** Heterogeneously structured electrospun composite scaffolds have performed well as ECM-mimetic tissue replacements in recent years. In light of this, we present an assessment of amnion derived multipotent stromal cell (aMSC) growth on hybrid polycaprolactone(PCL)-gelatin(G) scaffolds in terms of viability, infiltration depth and metabolic activity.

**Methods:** Four scaffold types were electrospun (150 µm thick) with different PCL:G ratios and fibre diameter distributions using vertical (V) and horizontal (H) orientations - PCL175V (175 mg/ml, homogenous), PCL125G50H (125:50 mg/ml, homogeneous), PCL125G50V (125:50 mg/ml, heterogeneous), PCL100G75V mg/ml (100:75 mg/ml, heterogeneous). A 7 day live/dead assay and a 15 day infiltration study (depths measured using confocal laser scanning microscope) with aMSCs from *Callithrix jacchus* were performed. Metabolic activity was measured by MTT assay (7 days). Additionally, gelatin loss during scaffold degradation (30 days) was measured by Raman spectroscopy.

**Results:** In general, PCL-gelatin blends perform better than unblended PCL. PCL125G50V shows the deepest cell infiltration on all days (76.3±7.1 µm by day 15). It is also the only sample that shows an increasing trend in metabolic activity from day 1 (0.0436±0.002) to day 7 (0.0996±0.009). Raman spectroscopy revealed a significant gelatin loss of 53.44±6.48 % in PCL100G75V while PCL125G50V showed no significant loss by day 15.

**Discussion:** A combination of heterogeneous fiber morphology and composite polymer content in electrospun scaffolds positively affects cell growth and infiltration. Considerable gelatin swelling during degradation can physically inhibit infiltrating cells. Therefore, choosing the right ratio of PCL:gelatin when designing such scaffolds is key.

**Acknowledgements:** Project funded by Deutsche Forschungsgemeinschaft (DFG) for Cluster of Excellence REBIRTH (EXC 62/3,62/4). We thank Dr Norman Liaw for the supervision of Sarah Kidwai from Göttingen.

O225

**POLYSACCHARIDE-BASED HYDROGELS FOR BONE TISSUE REGENERATION**M. Muhammad<sup>\* 1</sup>, C. Willems<sup>1</sup>, A. Liedmann<sup>1</sup>, A. Repanas<sup>1</sup>, G. Gallego-Ferrer<sup>2,3</sup>, T. Groth<sup>1,4</sup>

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**Objectives:** The main goal of this study was to synthesize polysaccharide-based hydrogel systems that favor bone regeneration. The hydrogel formation is driven by Schiff base crosslinking reaction between the amino functionalities of carboxymethyl chitosan (CMChi) and the aldehyde groups of the different oxidized polysaccharides resulting in an imine bond formation.

**Methods:** Different amounts of NaIO<sub>4</sub> were used in order to oxidize Alginate (ALG) and Hyaluronic acid (HA), forming reactive dialdehyde derivatives with different oxidation degrees. The amount of aldehyde groups was determined by: A) using Schiff's reagent and detected by a UV-Vis spectrometer (550nm) and B) acid base titration after reacting the aldehyde groups with Hydroxylammoniumchloride. Gel Permeation Chromatography (GPC) was used for molecular weight determination of the different polysaccharides. Biocompatibility of the products was tested using Qblue<sup>®</sup> assay and CFDA staining.

**Results:** Data obtained from both UV-VIS spectroscopy and titration show that the different polysaccharides have more aldehyde groups when high concentrations of NaIO<sub>4</sub> were used and the reaction times were extended. The weight average molecular weights of all the products were lower after the oxidation step. Products with high oxidation degrees showed low biocompatibility.

**Discussion:** ALG and HA with high oxidation degrees are required to prepare stable hydrogel systems and the dual utility of using CMChi and oxidized polysaccharides as both structural and bioactive component is promising for the formation of biocompatible hydrogel systems that can be used for bone tissue regeneration.

**Acknowledgements:** This work was supported by Deutscher Akademischer Austauschdienst DAAD (grant N<sup>o</sup>. 91605199) and Bundesland Sachsen-Anhalt in the frame of Leistungszentrum Chemie- und Biosystemtechnik (project N<sup>o</sup>. H01127247). The Spanish Ministry through the MAT2016-76039-C4-1-R project (including the FEDER financial support) is acknowledged.

O226

**A DIELECTRIC STUDY OF PIEZOELECTRIC FIBROUS MATS WITH ELECTRICAL SWITCHING EFFECT FOR NEURAL TISSUE IMPLANTS**A. Tretiakov<sup>1</sup>, V. M. Kapralova<sup>\* 1</sup>, N. T. Sudar<sup>1</sup>, O. Gryshkov<sup>2</sup>, B. Glasmacher<sup>2</sup>

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**Objectives:** Polyvinylidene fluoride (PVDF) and its co-polymer with trifluoroethylene (PVDF-TrFE) demonstrating biocompatibility and piezoelectric property are among the most promising biomaterials for nerve regeneration. Their blends with polydiphenylene phthalide (PDP) can improve nerve tissue regeneration due to combined electrical switching effect of PDP.

**Methods:** Electrospinning and film casting were used to produce porous electrospun fibre mats and thin films, respectively. SEM, DSC, RAMAN and FTIR spectroscopy, as well as piezoelectric measurements were performed to analyse morphology and physical-chemical properties of initial and final materials. A special measuring cell was designed to conduct dielectric spectroscopy measurements on samples with both deposited and pressed contacts.

**Results:** Electrospun PVDF-TrFE and PVDF-TrFE+PDP fibre mats and casted PVDF-TrFE, PVDF, PVDF-TrFE+PDP film samples were produced. The range of fibre diameter of 0.5-1.2 µm was obtained from SEM images. RAMAN (500-3500 cm<sup>-1</sup>) and FTIR (600-2000 cm<sup>-1</sup>) spectra were obtained for initial and final materials. The dependence of the samples capacity on the uniaxial pressure was measured. The frequency dependence of the capacitance and tangent of the dielectric loss angle tg(δ) at a constant uniaxial pressure has also been analysed.

**Discussion:** RAMAN spectrum of PDP+PVDF-TrFE fibre mats contains peaks from PDP and PVDF-TrFE, indicating that electrospinning method is suitable to produce PDP-containing PVDF-TrFE materials. Results of dielectric measurements showed that the presence of TrFE in co-polymer significantly reduces the value of the samples' capacitance. The capacitance of fibrous samples is much lower than that of films due to high porosity of fibre mats (>90%). TrFE-containing samples demonstrate high dielectric losses at low frequencies. The combined analysis of dielectric and piezoelectric properties is ongoing.

**Acknowledgements:** This work was supported by the DAAD project Strategic Partnership

**Biomaterials for Biomedical Applications**

O231

**DEVELOPMENT AND CONSTRUCTION OF AN IMPLANT SYSTEM TO PERFORM ALL INTERNAL BIFOCAL SEGMENTAL BONE TRANSPORT TO RESTORE LARGE BONE DEFECTS**D. Siebert<sup>1</sup>, H. Kistmacher<sup>2</sup>, S. Knigge<sup>1</sup>, B. Glasmacher<sup>1</sup>, C. Krettek<sup>3</sup>

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**Objectives:** Currently, there are no reliable internal treatment options for large segmental bone defects. The objective of this study is to develop an alternative treatment for a young male patient with a huge 180mm diaphyseal tibial bone defect, who was already recommended amputation.

**Methods:** A modular add-on system is developed for a commercially available motorized bone lengthening nail (Ellipse Technologies, USA). The system allows bifocal segmental transport of osteotomized bone segments. By this, the bone defect is filled from two sides. A wire rope hoist shall ensure that the bone can overcome the distance from both sides, which reduces the time of healing and the amount of implanted parts. A 3D-printed model of the patient's bone segments helps to

design the implant system itself and the handling which includes fixation, transport and explantation.

**Results:** The results show in accordance to previous studies that the developed implant system effectively transports both osteotomized transport bone segments. The wire rope hoist mechanism and the modular construction works as planned. The implant system can be explanted successfully.

**Discussion:** In this study, we present an individualized modular add-on implant solution. The advantage of the modular setup is that many parts can be manufactured in universal sizes. This method of bone reconstruction could help many patients, who suffer from large critical size defects.

O232

### PLASMA NANOFILMS AS BIOCOMPATIBLE INTERFACE FOR IMPLANTS

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**Objectives:** In the development of biocompatible materials for biomedical applications, the foreign body response is an important issue. The healing of surrounding tissue often interferes with the function of an implanted biomaterial. Events like protein deposition, hemostasis, inflammation, tissue repair, infections and the encapsulation of the functional part of the implant are the main cause of failure of the implanted device.

**Methods:** In this study biocompatible nanofilms are produced by means of a plasma polymerization process using a low-pressure magnetron-enhanced 15 kHz glow discharge. This process allows the precise control of the film nature and behaviour. The resulting hydrocarbon film has a thickness of a few nanometer and keeps therefore the inherent properties of the substrate material. Measurements on protein adsorption gave the possibility to tailor the thin films in the needed direction. This means to tailor a native secondary structure of in situ adsorbed proteins.

**Results:** The nanofilms were investigated using different surface analytical methods. Also the interaction in contact with different biological sample materials was tested in-vitro. The precise measurement of the adsorbed proteins indicated a native secondary structure of proteins on these surfaces. Different in-vivo sensor dummies which are in contact with blood and soft-tissue were coated by these nanofilms. The explanted sensors were kept free of any encapsulation by this coating. This stands in good correlation with the measurement of adsorbed proteins.

**Discussion:** The first in-vitro results of the adsorption of blood proteins indicated already a very biocompatible character of these nanofilms. The explanted sensors were kept free of any encapsulation by this coating. These coatings can open the door for many new applications in the field of new implants but also other biomedical products.

O233

### HEMOCOMPATIBILITY OF DOUBLE FILTRATION LIPOPROTEIN APHERESIS USING POLYETHERSULFONE VS. ETHYLENE-VINYL ALCOHOL COPOLYMER MEMBRANES

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**Objectives:** Clinical data on the hemocompatibility of membranes used in double filtration lipoprotein apheresis (LP) is virtually unavailable. The present trial compared the hemocompatibility of a recently introduced polyethersulfone (PES) based plasma fractionator membrane, FractioPES<sup> </sup> 200, to an ethylene-vinyl alcohol copolymer (EVAL) membrane during LP.

**Methods:** In a prospective, randomized, controlled, crossover trial, eight patients on routine LP were subjected to one treatment with PES plasma (0.6 m<sup>2</sup>, 3M PlasCure<sup> </sup> 0.6) and fractionation (1.9 m<sup>2</sup>, 3M SelectiCure<sup> </sup> H19) membranes and one control treatment using a set of EVAL membranes (0.5 m<sup>2</sup>, Asahi Plasmaflo OP-05W; 2.0 m<sup>2</sup>, Asahi Cascadeflo EC-50W). Intraindividual treatment conditions were kept identical. At defined times, samples were drawn at different sites of the extracorporeal blood and plasma circuit to measure white blood cell (WBC) and platelet (PC) counts, complement factor C5a and thrombin-anti-thrombin III (ATIII).

**Results:** With a nadir at 25 min, WBC in EVAL decreased to 34 % of baseline vs. 64 % at 20 min in PES (*P*<0.001). PC only marginally decreased over time with both membrane types. Maximum C5a in venous blood was 30.0±11.2 µg/L at 30 min with EVAL and 14.0±12.8 µg/L at 25 min with PES (*P*<0.001). Compared to PES (23.3±15.2 at 5 min and 16.9±12.3 at 20 min, resp.), highest C5a concentrations were found in plasma after the EVAL plasma (56.1±22.0 µg/L at 10 min; *P*<0.001) and fractionation filters (50.6±19.4 µg/L at 30 min; *P*<0.001). ATIII levels did not rise until the end of the treatment without differences between membranes. Regarding Lp(a), LDL and HDL removal, both membrane sets performed equally (PES, 69.8±5.7, 64.9±8.8, and 17.4±13.6 %, resp., vs. EVAL, 69.5±6.0, 65.2±6.9, and 18.2±7.3 %, resp.).

**Discussion:** Compared to EVAL, PES membranes are more beneficial with respect to the classical hemocompatibility of extracorporeal treatment procedures, namely leukocyte and complement system activation.

O234

### SARDINE ROE AS A NOVEL SOURCE OF BIOACTIVE AGENTS TO PRODUCE LIPOSOMES WITH ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES

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**Objectives:** Fish roe is a highly nutritional product with diverse beneficial effects for the human health. This work used sardine (*Sardina pilchardus*) roe to produce novel delivery systems presenting antioxidant and anti-inflammatory properties.

**Methods:** Methyl-*tert*-butyl ether extraction method was used to obtain lipid- and water-soluble compound rich phases. The fatty acids profile was analyzed by GC/MS. The identification of the hydrophilic compounds was performed by LC-HRMS. Lipids were used to produce large unilamellar liposomes (LUVs). The hydration of the lipid film was performed with PBS or sardine roe-derived aqueous phase. LUVs were characterized by their size, surface potential and morphology. The cytotoxicity of the extracts was evaluated using L929 cells. Their antioxidant activity was evaluated against different radicals, and their anti-inflammatory activity was assessed in the presence of LPS-stimulated macrophages.

**Results:** GC/MS analyses demonstrated the presence of several fatty acids, such as  $\omega$ 3 fatty acids. LC-HRMS analyses revealed the presence of numerous bioactive compounds (e.g. essential amino acids and gadu-sol). LUVs prepared with the aqueous phase presented a higher heterogeneity in terms of size than when they were prepared with buffer. Both LUVs formulations have a negative surface charge and a spherical shape. The extracts presented antioxidant activity against peroxy, hydroxyl and nitric oxide radicals. Biological assays indicated that the extracts are cytocompatible for the lowest tested concentrations and that they can inhibit the production of proinflammatory cytokines, which reveals their anti-inflammatory effect.

**Discussion:** Our sardine roe-derived delivery systems and compounds have both antioxidant and anti-inflammatory activities and thus they may be considered as a valid alternative for the treatment of inflammatory conditions.

**Acknowledgements:** Financial support from Foundation for Science and Technology (Portugal; PD/BD/113795/2015 and PTDC/CTM-BIO/4388/2014).

O235

### POLYELECTROLYTE MULTILAYER AS RESERVOIR FOR NOVEL CATIONIC LIPOSOMES AS POTENTIAL SURFACE COATING FOR MEDICAL IMPLANTS FOR OSTEOGENESIS

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**Objectives:** Cationic liposomes OO4 (N-(6-amino-1-[N-(9Z)-octadec-9-enylamino]-1-oxohexan-(2S)-2-yl)-N0-[2-[N,N-bis(2-aminoethyl)amino]ethyl]-2-hexadecylpropanidamide) and DOPE contain a higher amount of amino groups than classical lipids. A polyelectrolyte multilayer system (PEM) made of chondroitin sulphate (CS) and collagen type I (COL I) was prepared by Layer-by-Layer technique with liposomes embedded in the terminal layers for the controlled release of components to promote osteogenic differentiation for bone regeneration.

**Methods:** Characterization of the PEM was performed using ellipsometry. This technique was used to investigate the thickness on substrate coated with PEM of CS, liposomes, COL I as terminal layer. The layer growth behavior of PEM was studied using Surface Plasmon Resonance (SPR). The cell adhesion of C2C12 cells and the cellular uptake efficiency of the liposomes embedded onto PEM was evaluated using fluorescence staining and flow cytometry.

**Results:** The SPR results showed a linear growth with an increase in the angle shift corresponding to the adsorbed mass of PEM. The increase in thickness after liposome adsorption was significant. The cell studies indicated that cells seeded on terminal liposome layer showed a higher amount of cells on the surface. The population of cells that took up liposomes are higher in the absence of serum than in medium with FBS.

**Discussion:** The quantification of liposome uptake indicated that the population of cells that took up liposomes were higher in the absence of serum. The results showed the uptake efficiency of cationic liposomes embedded onto a PEM system for controlled release of hydrophobic and hydrophilic models compounds into the cells.

**Acknowledgements:** CONACYT-México, German Academic Exchange Service (DAAD) No. 50015952. German Research Society (DFG) project N. 396823779. The High Performance Center Chemical and Biosystems Technology Halle/Leipzig and supported by the European Regional Development Fund (ERDF).

O236

### MATRIGEL-MIMETIC BIOMATERIAL FOR HUMAN INDUCED PLURIPOTENT STEM CELLS

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**Objectives:** Human induced pluripotent stem cells (hiPSCs) possess an exceptional differentiation potential, as they are able to differentiate towards cells from all three germ layers. Owing to this capability, they are an attractive cell source for regenerative medicine applications and in vitro disease models. To date, coated substrates with Matrigel® and Geltrex™ have been providing promising platforms for hiPSC culture and differentiation. Besides many advantages, both substrates provide some drawbacks such as batch-to-batch variation and xenogeneic origin, which pose problems in potential clinical applications. To tackle these issues, a matrigel-mimetic synthetic biomaterial able to maintain hiPSCs in culture is required. Cell behavior is controlled by a complex set of biophysical (e.g. stiffness) as well as biological (e.g. growth factors) parameters. In this study, we want to develop a fully synthetic hiPSC-supporting hydrogel that allows investigating the influence of biophysical and biological parameters on hiPSCs.

**Methods:** The substrates were prepared via crosslinking starPEG and heparin molecules with different molar ratios. Substrates were biofunctionalized with two methods based on NHS ester binding to amine groups of laminins in different ratios. hiPSCs were seeded on substrates and the medium was changed every day. Cells were stained after 72 hours for pluripotency markers (OCT4-SSEA4).

**Results:** Functionalized substrates with storage modulus of 4kPa maintained hiPSCs culture. On the other hand, no adherent cells were observed on the substrates with a storage modulus of 1kPa and 10kPa.

**Discussion:** StarPEG-heparin hydrogels functionalized with laminin mixtures clearly represented an appropriate matrigel-mimetic growth substrate for the hiPSC culture and maintenance of their pluripotency. 3D cultures have already been shown to be superior to 2D and it is an outlook of this work.

**Acknowledgements:** The project is funded by Zentrale Innovationsprogramm Mittelstand (ZIM).

### Cells, Tissues and Organs

O241

### INVESTIGATION OF DECELLULARIZED LIVER TISSUE REGENERATIVE POTENTIAL

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**Objectives:** Tissue decellularization is one of the novel techniques in tissue engineering and regenerative medicine for obtaining extracellular matrix, which doesn't preserve mechanical properties of native tissue.

Modified by the extracellular matrix, silk fibroin scaffolds can combine high biocompatibility and suitable mechanical properties.

**Methods:** Wistar rats were used as liver donors. Decellularization of liver tissue was performed by sequential incubation with three solutions of 0,1% SDS containing Triton X-100 in the following concentrations: 1%, 2% and 3%. Livers were ground in liquid nitrogen and a certain fraction of microparticles was separated by precipitation and centrifugation. Silk fibroin scaffolds were prepared by a casting method using water as a solvent. Microparticle suspension was blended with fibroin solution before film preparation. Scaffold structure was investigated by scanning probe nanotomography (SPM) and scanning electron microscopy (SEM). The cytotoxicity of scaffolds, cell adhesion and proliferation were evaluated. Modified scaffolds were used as wound coatings in a full-thickness skin wound model.

**Results:** Microparticles with a size less than 5  $\mu\text{m}$  were prepared from decellularized liver tissue. Modified silk fibroin scaffolds were obtained. Sinuous and rough topography of the obtained matrix was shown. The produced scaffolds did not show cytotoxic effect. The cells adhesion was significantly higher on scaffolds modified with decellularized liver tissue microparticles in comparison with non-modified scaffolds. Modified silk fibroin scaffolds promoted skin wound regeneration by 30% in comparison with non-modified scaffolds.

**Discussion:** Regenerative potential of extracellular matrix as microparticles was shown. Decellularization of organs and tissues may be used in tissue engineering and regenerative medicine for increasing the biocompatibility of the artificial grafts.

## O242

### EFFECTS OF LOCAL ISCHEMIC PRECONDITIONING IN HEPATIC PORCINE MODEL.

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**Objectives:** Due to the growing demand for organs in liver transplantation, techniques for optimization of suboptimal livers are needed such as normothermic liver perfusion (PNT). In this way, hepatic reperfusion of ischaemic preconditioning (PI) still remains unclear. This study aims to analyze the haemodynamic and biochemical alterations produced after intraoperative PI on livers subjected to 6 hours of PNT. As a secondary objective, we aim to determine the effect of PI on liver function.

**Methods:** Eight minipig livers, randomized to control (n=4) and PI group (n=4), were perfused during six hours in a PNT circuit (34°C). The PI group was subjected to 10 minutes of ischaemia by means of a Pringle *manoeuvre* in the hepatic hilum, followed by 10 minutes of reperfusion. Biochemical and gasometric parameters were measured at baseline and hourly in perfusate. Pressures and flows in hepatic artery (HA) and portal vein (PV) were registered. For statistical analysis Spearman's rho test and Mann-Whitney U test was performed, considering  $p < 0,05$  as significant.

**Results:** There were significant differences in HA flow, adjusted to weight, doubling the control group (PI  $0,42 \pm 0,17$  ml/min/g Vs GC  $0,17 \pm 0,05$  ml/min/g;  $p=0,000$ ); no differences were found in PV flow. Total proteins (PI  $1,9 [1,7-2]$ g/dL Vs GC  $1,3 [0,95-1,5]$ g/dL;  $p=0,000$ ) and O<sub>2</sub> consumption rate (PI  $0,086 [0,055-0,113]$  ml/min/g Vs GC  $0,040 [0,017-0,060]$ ml/min/g;  $p=0,000$ ) were significantly increased in the PI group. There were no significant differences in AST, ALT, or GGT.

**Discussion:** The PI, as a previous maneuver to PNT, increases hepatic artery flow and O<sub>2</sub> consumption without altering cytolytic parameters in liver. This may improve hepatic functionality as a previous step in transplantation. Further molecular and histological study should be made to clarify our finding in PI on PNT.

## O243

### DEVELOPMENT OF A MULTI-LAYERED CRYOGEL BIOREACTOR WITH OPTIMISED FLUID DYNAMICS FOR BIOARTIFICIAL LIVER APPLICATION

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**Objectives:** Cryogels with improved non-fouling and cell adhesive surface properties were investigated as potential cell scaffold for BAL purpose. Cryogel monolith columns were used by other groups to fill the bioreactor chamber. However, device performance was not maintained for more than a few hours. Fluid dynamic measurements using a purpose-built  $\mu\text{PIV}$  setup with video post-processing were conducted in order to extrapolate the effect of flow inside the cryogel matrix to improve cell viability and avoid blood cell activation. Starting from PIV results, a multi-layered bioreactor composed of spaced cryogel discs was developed to maximise blood/hepatocyte mass-exchange. This study aimed to investigate whether the multi-layered design results in improved performance compared to the column version in terms of hepatocytes viability and functionality.

**Methods:** p(HEMA-co-MBA) cryogels were synthesised by cryogelation technique. Cryogels were then functionalised with alginate and RGD peptide and synthesised by solid phase method. Porous structure was analysed with SEM, confocal imaging and  $\mu\text{CT}$ . Non-fouling properties were investigated by protein absorption studies. Cell viability was assessed by MTT/ATP activity and live/dead imaging. Hepatocyte functionality in bioreactors was investigated by quantification of albumin and urea production using ELISA and colorimetric assay, respectively, over time.

**Results:** Synthesised cryogels possessed an open porosity with pore sizes of up to 100 $\mu\text{m}$  and an interconnected network of pores. Alginate helped preventing protein absorption from plasma. RGD peptide enhanced hepatocyte functionality. Cryogel perfused bioreactors maintained hepatocytes viability and functionality for up to 1 week.

**Discussion:** Multi-layered bioreactor design showed a significantly higher production of albumin and urea compared to the column version, suggesting an improved mass exchange between medium and cells. Also cell colonization and proliferation through the device were improved.

## O244

### GENERATION OF IMMUNOLOGICALLY INVISIBLE PORCINE PANCREATIC ISLET CELL CLUSTERS AFTER SINGLE CELL ENGINEERING AND ISLET REASSEMBLING TO SUPPORT XENOGRAFT SURVIVAL

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**Objectives:** Xenotransplantation of transgenic porcine pancreatic islets offers a promising alternative source to circumvent current limitations posed by the scarcity of allogeneic donors. However, either immune rejection or oxygen supply in immune protected encapsulated islets remains a major concern. To decrease xenogeneic immune responses, we have investigated the feasibility to generate tissue engineered SLA silenced islet cell clusters (ICC) from alpha-Gal knock out, CD46, CD55 and CD59 transgenic minipigs.

**Methods:** Pancreatic islets single cell suspensions were generated by enzymatic digestion of porcine ICCs. The single cells were silenced for SLA class I and II by lentiviral vectors encoding for Nanoluciferase as reporter gene and for short hairpin RNAs targeting beta2-microglobulin (shb2m) or class II transactivator (shCIITA), respectively. SLA transcripts were evaluated by real-time PCR and protein levels by flow cytometry and fluorescence microscopy analyses. Cell death was evaluated by Propidium Iodide staining. The effect of SLA class I silencing was evaluated in human T and NK cell cytotoxicity assays. SLA-silenced pancreatic beta-cells were then used to form new ICCs in stirred bioreactors.

**Results:** SLA class I silencing was designed to reach a level of up to 86% and class II by up to 64% on pancreatic islet cell monolayers. Silencing SLA expression did not affect cell viability and the insulin-producing beta-cell phenotype as indicated by Dithizone staining and levels of insulin production. Xenogeneic T-cell immune responses ( $p < 0.05$ ) as well as antibody-mediated cellular-dependent immune responses ( $p < 0.01$ ) were significantly decreased. Silencing SLA class I expression did not increase susceptibility to NK-cell cytotoxicity. In stirred bioreactors, tissue engineered islets showing the typical 3D-structure and morphology of ICC were assembled from SLA-silenced pancreatic cell suspensions to be used for transplantation in humanized mice as a first model.

**Discussion:** These data shows the feasibility to generate low immunogenic porcine ICC from transgenic pigs after single cell engineering and post-transduction islet reassembling that might serve as a robust alternative to allogeneic pancreatic islet cell transplantation.

O245

#### APHERETIC MEDICINE IN HEART TRANSPLANTATION: ANOTHER WEAPON IN OUR HANDS?

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**Objectives:** The improvement of immunosuppressive strategies have significantly reduced the risk and mortality of cellular rejection after Heart Transplantation (HTx). Recently, Humoral Rejection and antibodies have been recognized as a major problem in the management of recipients during the follow-up.

**Methods:** 23 patients out of more than 700 recipients undergoing HTx and 6 undergoing retransplantation required techniques of apheretic

medicine to manage Humoral Rejection episodes or to lead to transplant patients with Donor Specific Antibodies (DSA).

**Results:** In our experience 4 patients were treated with photopheresis with excellent tolerability, no adverse event and no rejection episodes. Photopheresis was added on top of standard immunosuppressive therapy in patients requiring minimization of calcineurins due to other clinical conditions. 19 patients experiencing humoral rejection were treated alternatively with Plasma Exchange (8 patients) or with immunoadsorption. Plasma Exchange was preferred in acute patients requiring removal of inflammatory factors other than antibodies while Immunoadsorption was better tolerated and adopted to reduce the metabolic impact and coagulative disorders when the patient was more stable.

**Discussion:** Apheretic Medicine has opened a new door and represents an efficient weapon in the armamentarium of an HTx center. The complexity of the management of this new disease in frail patients highlights the need for a multidisciplinary group establishing the right treatment for every patient.

O246

#### ANALYSIS OF PHOTOPHERESIS THERAPY WITHIN THE FRAME OF THE WAA REGISTER.

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**Objectives:** Data from the WAA apheresis registry was investigated regarding patients treated with extracorporeal photopheresis (ECP) as a tool to modulate immune response in various diseases.

**Methods:** 381 patients (34% women) had been treated with ECP for a total of 10668 procedures. The mean age was 48 years ( $\pm 18$ , range 3-81 years). Estimation of quality of life was made using grade 0 (suicidal) up to 10 (best ever) and health quality grade 1 (Bed ridden, ICU condition) up to 10 (athletic). Adverse events were analyzed. ANOVA comparisons were used.

**Results:** Most patients were treated due to graft versus host disease (GVHD,  $n=284$ ) and other hematological diseases ( $n=34$ ). Adverse events were registered in 5.8% of the first treatments and in 2.1% of the subsequent procedures. Severe adverse events were present in 0.4% of all procedures. No patient died due to the procedure. Tingling and stitching were the most common side effects. For those with GVHD the QoL was initially at a mean 6.3 ( $\pm 1.5$ , range 2-10) and improved significantly within 10 procedures and the Health Condition estimate improved significantly within 9 procedures and improved further with added procedures.

**Discussion:** Photopheresis is an established therapy with few side effects. The present data indicate that approximately 10 procedures are necessary to note significant effect to GVHD.

## Experimental and Computational Methods

O251

### INVESTIGATION OF THE ATTACHMENT OF ENDOTHELIAL CELLS TO A CELL PROBE FOR THE DIAGNOSIS OF CARDIOVASCULAR DISEASES

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**Objectives:** Circulating endothelial cells can be used to diagnose cardiovascular diseases. However, their concentration in the blood is very low (<100 per ml). A large volume of blood is needed, which should not be drawn from the patient. Instead a probe is inserted into the bloodstream of the patient to isolate as many cells as possible. The geometry of the cell probe is one of the key factors affecting cell deposition because its shape influences the flow conditions along the cell probe. The aim of this combined in vitro and in silico study is to find out the optimal flow conditions for cell deposition aiming to develop in future novel optimal cell probe shape.

**Methods:** In vitro experiments in a closed loop with endothelial cells from HUVEC cell line were carried out and the cell deposition as a function of the probe shape (4 probe types) and five different flow rates were investigated. The knowledge of the flow conditions at the cell probe was obtained by flow simulations by using CFD software STAR CCM+. By combining the experimental and simulation data with an iterative procedure, wall shear stresses, which seems to promote cell deposition, can be identified.

**Results:** It could be shown that the geometry of the cell probe has a much greater influence on cell deposition than the flow rates in the blood vessel. Wall shear stress around 0.4 Pa seems to be responsible for the optimal deposition of endothelial cells. However, this flow condition does not correlate with all flow rate experiments.

**Discussion:** The results of the combined in vitro – in silico study on deposition of endothelial cells on the existing cell probe design are promising. However, the used in vitro set up does not allow to extract maximal information from each test and should be redesigned.

O252

### EXPERIMENTAL COMPARISON OF TUG FORCE AND RADIAL FORCE OF LEFT ATRIAL APPENDAGE OCCLUSION DEVICES

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**Objectives:** In consideration of the recently published implant files, testing of medical devices has gained significant importance. Many medical implants are subject to normative testing during their regulatory approval process, such as stents, heart valves and blood pumps. No

testing norm or standard, however, exists for regulatory approval of left atrial appendage occlusion devices.

Therefore, this study aimed to establish *in-vitro* bench tests for LAA occlusion (LAAo) devices and compares the clinically most widely used devices.

**Methods:** Seven different LAA occlusion systems with device diameter ranging between 22 and 34 mm were tested regarding tug force and radial force resulting in a total of 24 devices. Radial force was assessed in a commercially available tester whereas tug force was evaluated in a novel in-vitro test setup consisting of bovine tissue.

**Results:** Significant differences in the mechanical properties of the different devices were observed. Radial force ranged between 8.6 N at maximum compression for the L'Ambre 2228 device and 0.1 N for the Occlutech 27 mm implant at minimum compression. A similar variability of mechanical properties was seen in the tug test results. Values ranged from 4.6 N to 0.4 N for the Wavecrest 22 mm and the Occlutech 24 mm device, respectively, at maximum and minimum compression.

**Discussion:** Large variations in mechanical properties were seen between the different devices. The study showed that device stability is more dependent on anchoring structures, such as hooks and barbs, than on radial force. A strong positive correlation between the number of anchoring structures per millimeter circumference of an occluder and its tug force was found ( $r=0.87$ ,  $p < 0.01$ ). The large variations in mechanical properties aggravate comparison of current LAA occlusion devices which underlines the need for standardized preclinical testing to prompt clinical compatibility.

O253

### STANDARD HEMOLYSIS TESTS: IS THE RESISTANCE'S DESIGN A PROBLEM?

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**Objectives:** Standardized hemolysis tests according to ASTM F1841-97 (2017) are the current gold standard for hemolysis evaluation. As part of the standard flow loop, a screw clamp is used to regulate the flow resistance in the circulations. Especially with extracorporeal pumps used for lung support, cardiopulmonary bypass application, or CO<sub>2</sub>-Removal, resistance has to maintain a pressure gradient of more than 500 mmHg. As a consequence, the screw clamp itself turns into a critical hemolysis hotspot, possibly overlying and biasing the hemolysis caused by the tested pump. Therefore, we present an alternative resistance causing only low hemolysis and at the same time maintaining ASTM testing specifications.

**Methods:** We developed one novel resistance and evaluated the hemolysis potential of the novel and one alternative in flow loops. The flow loops were set up according to the ASTM standard with three identical pumps (Deltastream® DP3, Medos AG), two with the novel and alternative resistances each and one with the state-of-the art resistance, the screw clamp. The loops were set to high pressure conditions present in cardiopulmonary bypass applications, ECMO or CO<sub>2</sub>-Removal. The loops operated for 6 hours with porcine blood ( $n = 6$ ). Blood samples were drawn hourly and free plasma hemoglobin was evaluated by means of photometric measurement.

**Results:** A new resistance for ASTM pump evaluation at high pressure regimes with little impact on hemolysis was developed. The resistance is



easy to integrate into the recirculation loop and can be adjusted continuously. In-vitro blood trials revealed lower hemolysis for the new resistance compared to the current gold standard.

**Discussion:** Extracorporeal continuous flow blood pumps with transfemoral cannulation work against high pressure at low flow. Up to now, hemolysis evaluation of the pumps is biased by hemolysis caused by the flow loop resistance. Our newly designed resistance causes less hemolysis, thus allowing for more meaningful and reliable hemolysis test results.

O254

#### A COMPUTATIONAL MODEL OF CHEMICAL AND MECHANICAL PLATELET ACTIVATION AND AGGREGATION

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**Objectives:** Thrombotic deposition is a major consideration in the development of implantable cardiovascular devices. Recently, it has been demonstrated that fluid mechanical shear micro-gradients play a critical role in thrombogenesis. The goal of the present work is to develop a predictive computational model of platelet activation and deposition that can be used to assess the thrombotic burden of cardiovascular devices. We have developed a comprehensive model of platelet-mediated thrombogenesis which includes platelet transport in the blood flow, platelet activation induced by both agonists generated at sites of vascular injury and shear micro-gradients, kinetics and mechanics of platelet adhesion, and changes in the local fluid dynamics due to the growth of a thrombus.

**Methods:** A 2D computational model was developed using the multiphysics finite element solver COMSOL 5.3a. The model can be described by a coupled set of convection-diffusion-reaction equations, and it comprises 7 species: resting and activated platelets, agonists that induce thrombosis, and an anticoagulant agent. Platelet adhesion at the surface was modeled *via* flux boundary conditions. Using a moving mesh for the surface, thrombus growth and consequent alterations in blood flow were modeled. In the case of a stenosis, the notions of shear stress-induced platelet activation in the acceleration zone and platelet deposition in the expansion zone downstream of the stenosis were studied.

**Results:** The model provides the spatial and temporal evolution of thrombosis in the flow field. The computed density of platelets adherent to the surface was validated against experimental data. The results confirm the importance of considering both mechanical and chemical activation of platelets.

**Discussion:** The developed model represents a potentially useful tool for the optimization of the design of the cardiovascular device flow path.

**Acknowledgements:** The present work was supported in part by the startup Corwave.

O255

#### ARTIFICIAL GENERATION OF SHEAR INDUCED THROMBI FOR MODELLING OF LVAD PUMP THROMBOSIS

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**Objectives:** Ventricular assist devices are a commonly used therapy for end stage heart failure patients. Pump thrombosis is one of the most common problems in patients supported by an LVAD. Two types of thrombus can be distinguished: white thrombi and red thrombi. The only available option to combat a PT is a lysis therapy or a pump exchange. The objective of the presented study is to develop a relevant white pump thrombus model to promote development of these concepts.

**Methods:** We developed an in vitro mock circulation system. The artificial blood circulation is provided by a HVAD. The HVAD pumps 80ml of human blood through the silicone loop. Parameters for inducing the white thrombus are high shear stress (4000 revolutions per minute), a rougher surface of the rotor, and an added activator of the intrinsic coagulation cascade. After two hours, the HVAD-system was checked for a white thrombus. Generated thrombi were stored for subsequent analysis (scanning electron microscopy and mechanical stability).

**Results:** Thrombus generation was reproducible in number and geometry. After ten test runs, 23 thrombi were detected. All these generated thrombi had a minimal size of 12 mm<sup>2</sup>. The developed thrombi are very similar in structure compared to white patient thrombi explanted in clinic. The scanning electron microscope showed a fibrin net on the surface of all tested thrombi (n=5). All tested thrombi samples (n=5) resist high strength (max. 1.029 MPa) in compression tests, which is typical for white PT.

**Discussion:** The size of the thrombus should be increased for special investigational needs. A protocol was developed for reproducible and reliable production of white, shear induced thrombi for lysis therapy investigations. In addition, the similarity (biological structure, mechanical stability) to explanted patient thrombi was shown. However, additional analyses should be performed for further characterization of the artificial thrombi.

O256

#### FLUID-STRUCTURE INTERACTION DURING EX VIVO PLATELET PRODUCTION

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**Objectives:** Ex vivo platelet production in microfluidic bioreactors is a promising alternative to platelet donation for transfusion therapy. Platelets are formed by fragmentation of large bone marrow cells called megakaryocytes (MK). Hydrodynamic forces play a major role in the formation of platelets. In this work, we design microfluidic chips where isolated MK are exposed to hydrodynamic forces and we characterize their elongation and fragmentation.

**Methods:** Polydimethylsiloxane chips are fabricated using standard soft lithography protocols and sealed to a glass slide. The 50 µm deep rectangular chambers contain rows of 30 µm-wide adhesive pillars. Human CD34+ cells isolated from umbilical cord blood are differentiated in vitro for 12 days to yield mature MK and infused with a concentration of 2x10<sup>5</sup> /mL into the chips at a wall shear rate of 1800s<sup>-1</sup>. Their elongation and fragmentation is monitored by videomicroscopy. Images were analysed using ImageJ.

**Results:** We perform a spatio-temporal analysis of MK elongation and show that platelet release is always preceded by a remodeling of the cell that spans over about 20 minutes, followed by a local, sudden increase in elongation velocity (5-fold increase in the 10 seconds prior to fragmentation). The amplitude of these variations is much larger than the spatial and temporal variations in the surrounding flow field.

**Discussion:** Earlier studies have shown that dynamic conditions for in vitro platelet production not only accelerates the process but also enhances the quality of released platelets. Here, we show that MK

elongation and fragmentation, though accelerated by the flow, are not solely governed by fluid-structure interactions. It seems that other mechanisms are involved in the regulation of platelet release, preventing it to occur before the platelet is fully functional.

**Acknowledgements:** This work was supported by Région Hauts-de-France and the European Union (FORPLAQ project, FEDER program), and by the French National Research Agency (TRIMEP project).

## Cell Stimulation

O261

### POLARIZATION OF PIEZOELECTRIC POLYVINYLIDENE FLUORIDE VIA ELECTROSPINNING FOR ACTIVE FILTER MEMBRANES

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**Objectives:** The piezoelectric effect of polyvinylidene fluoride (PVDF) is a promising property to be considered when controlling the diffusion of ions through membranes. This study examines the impact of different solvents and their ratios on the piezoelectric  $\beta$ -phase of PVDF and on the diffusion rates of fiber membranes.

**Methods:** Fiber mats were polarized via electrospinning using 20w% PVDF solutions with solvent mixtures of dimethylformamide (DMF) or dimethylacetamide (DMAc) as first solvent and acetone or methyl acetate (MeOAc) as second solvent in different ratios. Conversion of non-polar  $\alpha$ -phase into polar  $\beta$ -phase was evaluated via Fourier-transform infrared spectroscopy (FTIR). Diffusion experiments were performed by measuring changes in conductivity of deionized water ( $1.5\mu\text{S}/\text{cm}$ ) for 150min under static and cyclic loading conditions.

**Results:** Polymer solutions showed a non-Newtonian behavior. An increasing acetone ratio led to decreasing densities in PVDF solutions. Densities remained unchanged for all MeOAc ratios. FTIR results for MeOAc-solubilized PVDF fiber mats showed lower intensity of  $\alpha$ -phase peaks at  $762\text{cm}^{-1}$  as compared to acetone solubilized ones. The intensity of the characteristic  $840\text{cm}^{-1}$  polar  $\beta$ -phase peak rose with a higher ratio of the second solvent. Diffusion experiments with piezoelectric PVDF membranes ( $n=5$ ) inhibited sodium chloride diffusion under static conditions ( $67\mu\text{S}/\text{cm}$ ) while induction of the direct piezoelectric effect allowed for increased diffusion ( $533\mu\text{S}/\text{cm}$ ).

**Discussion:** Solvent properties and ratios influence the intrinsic properties of PVDF solutions for electrospinning and consequently affect  $\beta$ -phase conversion. Especially, MeOAc in combination with DMF or DMAc has a positive effect on piezoelectric  $\beta$ -phase conversion. With suitable process parameters, piezoelectric fiber mats can be produced for active filter membranes. Ongoing experiments aim to investigate their molecular weight cut-off properties.

**Acknowledgements:** Thanks to A. Vogt and K. Hölting.

O262

### ELECTRICAL STIMULATION OF CELLS GROWTH ON CARBON NANOTUBE- AND ORGANIC COMPOUND-BASED SUBSTRATES

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**Objectives:** This investigation discusses the method of electrical stimulation of human umbilical cord-derived mesenchymal stem cells (MSC) and skin fibroblasts (FB) growing on substrates composed by carbon nanotubes and organic compounds.

**Methods:** Substrates were produced from a water dispersion of single-walled carbon nanotubes and organic compounds (serum albumin, collagen, chitosan) and formed by evaporation of the liquid component using infrared laser radiation. Laser exposure stimulated the formation of conductive nanotube clusters responsible for electrical signal transmission to the cells, and organic compounds improved cell adhesion due to the presence of cell membrane receptors sensitive to matrix proteins and aminosugar molecules. An aseptic system for electrical cell stimulation included an electric pulse generator, culture plate with cells with a bread-board and electrodes made of surgical steel with gold plating. Cells were stimulated for 48 hours with pulsed electric signal (60-200 mV amplitude, 1 ms pulse duration, 1 s pause) directly inside the  $\text{CO}_2$ -incubator.

**Results:** The effects of electrical stimulation on the cell proliferation were estimated by MTT-test. It was found that both MSC and FB are sensitive to the stimulus, but the most prominent increase of cell proliferation was detected at 80 mV pulse amplitude. In these conditions, a 13 and 16 percent augmentation of cell growth rate was obtained for MSC and FB, respectively. Similar results (the formation of denser cell monolayer) were achieved by fluorescent microscopy of cell nuclei stained by ethidium bromide and fluorescein diacetate. No pathological changes in cell morphology were observed.

**Discussion:** The obtained results suggest that MSC and FB growing on conductive substrates are sensitive to electrical stimulation. This observation can be explained by the activation of potential-controlled ion channels and the stimulation of metabolic processes that accelerate cell growth.

O263

### DESIGN OF A PERSONALIZED GENERATOR WITH FREQUENCY TUNING FOR INDUCTIVE POWERING OF ARTIFICIAL ORGANS

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**Objectives:** The purpose of this work is to design and create a high efficiency inductive powering unit (IPU) for a wide range of implantable devices and artificial organs (AO).

**Methods:** A possible way to realize wireless powering is to use inductive energy transfer systems. To maximize overall system efficiency class E power amplifier is widely used due to its 100% efficiency in the ideal case. But maximum efficiency can be achieved only if input and output system parameters will remain constant during IPU operation. Inductive coils have nonzero distance between them because of patient's tissue thickness. It is worth noting that tissue thickness is different for every patient: the difference may be several millimeters. Moreover, this value can change during IPU operation as a result of patient's motor activity or tissue edema which will lead to PA detuning and decreasing efficiency. To avoid PA's detuning, frequency tuning systems can be used. There are two approaches for tuning systems that can be found in the literature. The first uses voltage and/or current levels of important circuit nodes to estimate system status. This method is good and quite simple, but requires external control signals that means additional power losses and

decreased efficiency. The other method uses the phase of the voltage signals from important nodes and consumes less power. The second method was chosen by authors to design a tuning system.

**Results:** During this work, a calculation method for PA and the frequency tuning system were designed. The experimental prototype was created. The authors of this article obtained an overall system efficiency of more than 60 % for three different coil sets and output power from 0.7 W to 1 W for same coil sets and range of axial misalignments from 10 to 20 mm.

**Discussion:** The high efficiency inductive powering system for AO has been designed and tested during this work.

**Acknowledgements:** This study was supported by the Ministry of Science and Higher Education of the Russian Federation (agreement № 14.579.21.0144, id RFMEFI57917X0144).

O264

#### EVALUATION OF THERMAL DAMAGE TO HUMAN BIOLOGICAL TISSUE DURING THE OPERATION OF A WIRELESS ENERGY TRANSFER SYSTEM USING THE FINITE ELEMENT METHOD

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**Objectives:** The aim of this work was to study the influence of the constructive and personal characteristics of the wireless energy transfer system on the temperature distribution in biological tissues. The basic parameters of the transcutaneous energy transfer system, such as transmitted power, efficiency and displacements, were taken into account. The patient's personal characteristics, such as the thickness of biological tissue layers, were also taken into account. To obtain data on the level of thermal damage, Henriques and Moritz study was used.

**Methods:** The temperature data was obtained using the numerical solution of a bioheat equation by the finite element method. This article mathematically evaluates thermal injury based on Henriques and Moritz study, in which damage can be represented as the speed of a chemical process.

**Results:** The temperature distributions in the tissues corresponding to the following types of displacements were obtained: axial, lateral, angular and also for the case where there are no displacements. Also the temperature distributions in tissues with different structures were obtained. Further, the temperature values at the maximum heating points were used to estimate thermal damage to the tissues.

**Discussion:** It was established that when a wireless energy transfer system is operated without displacements, the severity of thermal injury does not exceed the threshold corresponding to a first-degree burn. It was also found that the patient's personal characteristics are influenced by the temperature distribution in the case when a change in the thickness of a layer of biological tissue occurs in the region of the nearest heat source.

**Acknowledgements:** The results of this work were obtained within State Assignment of the Ministry of Education and Science of the Russian Federation (No. 12.2339.2017/ 4.6).

O265

#### INCREASE IN SPATIAL FREEDOM OF COUPLERS ORIENTATION IN INDUCTIVE POWERING UNIT FOR IMPLANTABLE MEDICAL DEVICES

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**Objectives:** The goal of this work was to study methods that will allow increase in spatial freedom of couplers orientation in inductive powering unit for implantable medical devices. An inductive energy transfer system is considered, therefore the freedom of relative orientation of the transmitting and receiving coils must be optimized.

**Methods:** The design procedure of inductive powering unit (IPU) consists of two main steps: design of the power amplifier and optimization of an inductive link. To account for the patient specifics, such as tissue thickness near the implantation site, a number of different couplers were simulated using finite element modeling. Effect of coils geometrical parameters on mutual inductance and inductive link efficiency was examined. Class E power amplifier was chosen as a driver for the transmitting coil. Capacitors in the amplifier loading network were tuned to achieve zero-voltage switching (ZVS). A patient's everyday activity, including walking and even breathing, leads to misalignment of the transmitting and receiving coils, and, as a result, detuning of the amplifier from ZVS and increased losses. Feedback schemes were studied to compensate the misalignments effect on the amplifier.

**Results:** A self-oscillating IPU with class E amplifier was designed that provides stable output power about 0.5 W for the distance between the couplers in range 10-20 mm and the lateral distance up to 20 mm. The use of self-oscillating circuit in the transmitter increases spatial freedom of the transmitting and receiving coils corresponding to ZVS.

**Discussion:** Inductive link geometrical parameters optimization and implementation of self-oscillating class E driver the IPU contributes to stable output power and efficiency of IPU. As a result, less intervention from the patient and physician is required, and patient quality of life is increased

**Acknowledgements:** This study was supported by the Ministry of Science and Higher Education of the Russian Federation (agreement № 14.579.21.0144, id RFMEFI57917X0144).

O266

#### WIRELESS POWERING OF ARTIFICIAL ORGANS: OVERCOMING CHALLENGES

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**Objectives:** The aim of this work is to provide a general overview of the design challenges in the wireless powering of artificial organs, to develop a general design strategy for inductive powering units (IPU), and to design a prototype IPU for mid-power application with high-stable output characteristic.

**Methods:** Artificial organs can benefit greatly from inductive wireless powering, but there are several major design challenges which must be overcome. The high energy transfer efficiency must be ensured. The IPU must tolerate coils misalignments. Tissue overheating must be avoided. Biocompatible materials must be used. Size and weight restrictions must be taken into account. Finally, patient body specifics should be considered. We have developed a new general design strategy in which all mentioned problems are addressed. The main feature is that we simultaneously optimize the inductive link and the schematics of the transmitter and the receiver and take the body specific elements (the thickness of the skin and the fat layer) into account. We have developed strictly formal procedures which can be implemented using software in computer-aided design procedures.

**Results:** In order to test and verify our approach we have designed a prototype IPU. The design goal was set as follows: IPU must ensure output power in the range 0.45...0.55 W for the axial distance between the coils in the range 10...20 mm and the lateral misalignments up to 20 mm.

The design goal was achieved. Several coil couples were used to adjust optimal energy transfer distance to the expected implantation depth. Measurements were performed in the air as well as with the samples of “skin-fat” biological objects were performed.

**Discussion:** We have developed and tested design strategy for biomedical IPU which can provide reliable design adjusted to the various requirements and the specific of the patient’s body.

**Acknowledgements:** This study was supported by the Ministry of Science and Higher Education of the Russian Federation (agreement No 14.579.21.0144, id RFMEFI57917X0144).

## Novel Cardiac Devices

O311

### THE REINVAD LVAD – SMART TECHNOLOGY TO ENHANCE LONG-TERM CIRCULATORY SUPPORT THERAPY

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**Objectives:** Despite the growing acceptance of LVAD therapy, concerns relating to adverse events have been raised. Fields such as implantation techniques, patient management and patient selection pose opportunities to advance outcomes, however, new and enhanced device technology, such as the ReinVAD LVAD, will have to contribute to improve and grow the therapy.

**Methods:** The ReinVAD GmbH was founded as spin-off company of the Helmholtz-Institute of the RWTH Aachen University, Germany, and leads the development project to regulatory approval and clinical application. The core technology is a miniaturized, 3<sup>rd</sup> generation, centrifugal blood pump. It is the result of a multidisciplinary research and development program equipping the system with features that will improve outcome for bridge to transplantation and especially destination therapy patients.

**Results:** Chronic trials typically up to 90 days in a sheep confirm the capabilities of the system with excellent resistance to thrombogenicity since no design related thrombus could be found despite ceased anticoagulation after 6 weeks. Implemented features are a pump for partial and full support with flow up to 10 l/min, a fully integrated flow sensor, an implantable driveline connector, a highly pressure-sensitive pump characteristic, an automated speed controller and a user-friendly one-piece extracorporeal system. The sensor provides real flow measurements. The driveline connector ensures easy exchange in case of infections and renders the system ready-for-TET. The pump pressure sensitivity, among other effects, leads to increased exercise capacity and arterial pulsation. The controller ensures that the pump always operates at its optimal speed. The one-piece extracorporeal system reduces use errors and improves satisfaction with this component of everyday life.

**Discussion:** The results indicate that the ReinVAD LVAD will be an effective system to enhance long-term circulatory support therapy.

**Acknowledgements:** The authors thank the Erich and Hanna Klessmann foundation, Germany.

O312

### CORWAVE LVAD: A PHYSIOLOGIC, PULSATILE-FLOW WAVE-MEMBRANE PUMP

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**Objectives:** Corwave LVAD is being developed to employ gentle oscillation of a membrane to propel blood, based on the wave motion of swimming fish. The pump output can be readily tuned by adjusting membrane oscillation frequency and magnitude. The purpose of this project was to increase the hydraulic efficiency, implement a physiologic pulsatility control algorithm, and confirm performance in animal implants.

**Methods:** The fluid path of the pump was simulated by Fluid-Structure-Interaction (FSI) computational fluid dynamic analysis in COMSOL. Membrane size, oscillation frequency, oscillation magnitude, and the blood flow path were modeled and refined to improve hydraulic performance and eliminate areas of flow stagnation. Pumps were then tested in blood analogs and blood in mock circulation loops and in vitro hemolysis testing. Non-hermetic prototype pumps were implanted in a total of 25 sheep for acute and chronic implants.

**Results:** Design simulations and individual component testing resulted in a pump which can generate 6+ LPM of blood flow against physiologic pressures with maximum shear rates orders of magnitude lower than those of rotary blood pumps. Mock loop testing demonstrated the pumps have “flat” HQ (pressure vs. flow) curves. Three control methods were developed: fixed, asynchronous pulsatile, and synchronous pulsatile. Pulsatile modes generated  $dP/dt > 400$  mmHg/s using sensorless detection of native ventricle systole. Animal implants demonstrated low hemolysis and an absence of renal infarcts, but were limited to about a week in duration due to the non-hermetic sealing of the pumps.

**Discussion:** Application of robust computational simulation and bench top testing have produced a unique new kind of VAD, offering the flow capacity and reliability of rotary pumps, but adding physiologically relevant pulsatility without excessive shear rates. Future efforts will implement full hermeticity to extend animal study durations and confirm von Willebrand Factor compatibility.

O313

### INVESTIGATION OF THE EFFECT ON BLOOD OF ROTOR PUMPS SPUTNIK-1 AND SPUTNIK-2

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**Objectives:** A major part of developing rotary blood pumps requires the optimization of hemolytic properties of the entire pump. The aim of this project was to compare blood flow simulation in the first and second generations of the ventricular assist device Sputnik and to evaluate its effect.

**Methods:** Studies of the flow field were conducted with commercial CFD software ANSYS FLUENT 16.0, which uses a finite-volume approach. Scalar shear stresses were calculated for both pumps. The pumps were compared in terms of volumes subjected to certain viscous shear stress thresholds, below which no trauma was assumed (von Willebrand factor cleavage: 9 Pa, platelet activation: 50 Pa, and hemolysis: 150 Pa), associated residence times and recirculation zones.

**Results:** Volume fractions of shear stress above 9 Pa, 50 Pa, 150 Pa were received: Sputnik-1 had 7.69 ml, 2.58ml, 0.74 ml, Sputnik-2 – 3.41 ml, 1.07 ml, 0.22 ml. The sum of the cell residence times at these shear stresses is 0.5095 s for the Sputnik-1 and 0.2019 s for the Sputnik-2. The volumes of recirculation zones were obtained, the value is 4.36 ml for the Sputnik-1 and 1.72 ml for the Sputnik-2.

**Discussion:** The second generation of the VAD Sputnik have better flow characteristics than first. In the operating point, the volume fraction of the shear stresses is less, the residence time is less and the volume of recirculation zones is less.

**Acknowledgements:** This study was supported by the grant from Russian Science Foundation No. 18-79-10008.

O314

#### CHARACTERIZATION OF HEMODYNAMIC AND ENERGETIC PERFORMANCE OF THE REALHEART™ TOTAL ARTIFICIAL HEART WITH A HYBRID CARDIOVASCULAR SIMULATOR

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**Objectives:** The aim of this work is to investigate the performance of the RealHeart™ total artificial heart (TAH) with a hybrid cardiac simulator (HCS).

**Methods:** The HCS, developed at the Nalecz Institute of Biocybernetics and Biomedical Engineering, is a computational-hydraulic model of the cardiovascular system. Four hydro-numerical interfaces reproduced the pressure/flow profiles of atria, pulmonary artery, and aorta hydraulically, while the rest of the circulation was numerical. The TAH was connected accordingly. The TAH is a pulsatile 4-chamber device whose left and right sides can operate independently, by changing pulse rate (PR) and stroke length (SL). The TAH was tested on the HCS at low/medium operating points: PR 60/80/100/120 bpm; SL 20/22/24/26 mm; systole duration ratio 0.4 of the TAH cycle. Systemic resistance was changed between 0.8 and 1.2 mmHg/(ml/s), pulmonary resistance was changed between 0.1 and 0.2 mmHg/(ml/s). Systemic and pulmonary compliances were set to 1.4 ml/mmHg.

**Results:** The independent tuning of PR and SL permits fine regulation the TAH flow on the left and right sides. As an example, for a peripheral resistance of 1.0 mmHg/(ml/s) the flow ranged from 2.7 l/min (PR=60,SL=20mm) to 7.0 l/min (PS=120,SL=26mm). For the investigated PRs and SLs, power consumption on the left pump ranged between 1.3 and 10.0 Watts and on the right pump between 0.9 and 6.0 Watts. The TAH provides a pulsatile flow, whose waveform depends on the SL and systole duration. This induced a pressure pulsatility ranging from 10mmHg (PR=60,SL=20mm) to 38 mmHg (PR=120,SL=26mm) in both the aorta and pulmonary artery.

**Discussion:** The HCS is a flexible test bench to evaluate the TAH performance in different hemodynamic conditions. The tuning of PR and SL on the left and right side of the TAH, independently, offers flexibility in regulating cardiac output and in managing the left/right flow balance. Power consumption is in the range suitable for clinical applications.

**Acknowledgements:** Frans Van de Werf scholarship, C14/18/080 project.

O315

#### FULLY IMPLANTABLE CENTRIFUGAL VENTRICULAR ASSIST DEVICE DESIGNED FOR MINIMALLY INVASIVE TECHNIQUE

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**Objectives:** Designing new ventricular assist devices to couple two major challenges in the field: less invasive intervention and reducing side effects was our objective.

**Methods:** Computer-aided design and computational fluid dynamics were performed with Solidworks 2017 to design and test several new bearingless centrifugal ventricular assist devices. Fused deposition modeling was used to build the models and subcutaneous implantation was simulated and transcutaneous power transfer was tested.

**Results:** Two bearingless, fully implantable ventricular assisted devices were developed. The first is a radial flux motor fully magnetically levitated ventricular assist device. The second is an axial flux hydro-magnetically suspended rotor ventricular assist device. Both devices contain an internal rechargeable battery, power transfer coil, electronic circuit and have a cylindrical shape with 15-20 mm height and 100 mm diameter. The flow is 3 l/min at 135 mmHg is achieved and efficiency of power transfer of 80% with a 5 mm gap. The devices are implantable in a pectoral subcutaneous pocket with the inflow connected to left atrium and the outflow to subclavian artery in a fashion pioneered in this field by CircuLite Synergy.

**Discussion:** Both devices came with innovations in motor and pump design that set the foundation to build the entire system in a single body low height cylindrical shape, optimal for subcutaneous implantation and by this with good transcutaneous power transfer efficiency.

O316

#### LEFT VENTRICULAR ASSIST DEVICE IMPLANTATION VIA LEFT THORACOTOMY AND UPPER HEMISTERNOTOMY: LONG-TERM FOLLOW-UP OF 111 PATIENTS

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**Objectives:** The epidemiology of heart failure (HF) is a rising medical burden with an estimated growing rate of 46% from 2012 to 2030. Left Ventricular Assist Devices (LVAD) and minimally invasive techniques are nowadays key therapies to guarantee optimal treatments for each HF patient. So far, clinical experience with minimally-invasive LVAD surgery showed promising short-term results. The aim of this study is to evaluate the long-term results of the first series of patients who received minimally-invasive LVAD surgery at our institution.

**Methods:** We reviewed the short and long term outcomes of 111 end-stage HF patients who received an LVAD implantation (HVAD, HeartWare, Medtronic, USA) at our institution between 2011 and 2014. All patients underwent an upper hemisternotomy and left-sided anterolateral thoracotomy with cardiopulmonary bypass.

**Results:** Between 2011 and 2014, 111 minimally-invasive LVAD implantations were performed at our institution (78% male, 22% female; mean age 52 ± 4; dilated cardiomyopathy 42.6%; ischaemic cardiomyopathy 44.4%, other aetiologies 13%). Postoperative bleeding incidence was 9.6% leading to a low amount of applied packed red blood cells (mean: 4.9 rbc), short ICU stay (mean 9.3 days), and a low incidence of right heart failure (4.6%). Thirty-day mortality was 5.2%, 90-day mortality 9.0% and 1-year survival 88%. Long-term follow-up (median 6.4 years) demonstrated a mortality of 51.4%, a transplant rate of 16.4%, and an explant rate of 1.7%. 15.8% of patients underwent pump exchange and 30.3% of patients had still an ongoing LVAD therapy at the time of follow-up.

**Discussion:** Minimally-invasive LVAD implantations are proven to be safe and associated with a lower perioperative complication and mortality rate. By using an upper hemisternotomy with an anterolateral thoracotomy, the intra-hospital outcomes showed promising results impacting also the long-term follow-up which presented an excellent percentage of ongoing LVAD therapies.

## Cardiovascular Biomaterials

O321

### ACCURACY OF INTRAVASCULAR ULTRASOUND TECHNIQUE FOR NATIVE AORTIC ANNULUS MEASUREMENT IN PATIENTS UNDERGOING TAVI: FIVE-YEAR EXPERIENCE.

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**Objectives:** TAVI is the treatment of severe aortic valve stenosis in elderly patients or in patients with too high risk for conventional surgery. CT-scan is mandatory for valve sizing. It requires injection of contrast medium that may cause or increase kidney failure. The study aim was to establish the accuracy of intravascular ultrasound (IVUS), a no contrast medium technique, for measurements of native aortic valve in patients undergoing TAVI. Values by IVUS were compared to CT-scan ones in the same patients.

**Methods:** From June 2014 to January 2019 137 consecutive patients (74 M, mean age 82.7±5.6 years) (Logistic EuroSCORE 22.7±14.9%; STS score mortality 21.3±13.7%) undergoing TAVI through femoral access were enrolled. Each patient had high resolution angio-CT for these measurements: diameter, perimeter, and area of the aortic annulus. In all patients, during procedure and before prosthetic valve implantation, a manual IVUS pullback, from left ventricular outflow tract to ascending aorta, was performed by 7F IVUS probe. On the recorded IVUS pullback, a second operator blind to CT measures sized minimum and maximum diameter, perimeter and area of the aortic annulus. In order to assess inter-rater concordance and agreement between CT and IVUS data, Bland-Altman analysis and Pearson's correlation were applied. A p value <0.05 was statistically significant.

**Results:** A very strong correlation was found between the measures. Maximum diameter: CT 2.5±0.3 vs IVUS 2.6±0.3 cm; r=+0.985; p<0.01. Minimum diameter: CT 1.7±0.1 vs IVUS 1.6±0.1 cm; r=+0.945; p<0.01. Area: CT 0.9±0.2 vs IVUS 0.8±0.3 cm<sup>2</sup>; r=+0.962; p<0.01. In 133 patients measurements by IVUS and CT-scan would have led to select a valve of the same size. In the remaining 4 cases we found a mismatch.

**Discussion:** These results prove the accuracy and reliability of aortic annulus measurements by IVUS compared to those by CT-scan. Sizing of the aortic annulus by IVUS only may be considered in patients likely to develop kidney failure due to contrast medium injection.

O322

### STERILE PERI-GRAFT ABSCESS FORMATION FOLLOWING ASCENDING- HEMIARCH AORTIC REPLACEMENT: A WORD OF CAUTION FOR USAGE OF BIOGLUE TISSUE SEALANT

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**Objectives:** Many different tissue sealants are available on the market. BioGlue tissue sealants are produced by a bovine serum albumin treated intra-operatively with glutaraldehyde. Here we report a sterile peri-graft abscess formation, possibly caused as a reaction to the liberal use of BioGlue.

**Methods:** A 54 years-old male patient underwent ascending aortic and hemiarch replacement for acute type A aortic dissection. Exceptionally, the anastomotic suture line was reinforced by application of BioGlue tissue sealant. The initial post-operative course was uneventful. After transfer to the general ward on day four the patient developed fever and an inflammatory syndrome. A broad spectrum antibiotherapy was started for a suspicion of pneumonia. In the following chest CT scan a suspicious peri-graft fluid collection was present. The patient underwent a surgical revision for acute mediastinitis rule out. During the procedure a whitish-milky liquid was found that differed macroscopically from a classical abscess. The chest was left open with a negative-pressure wound dressing. The patient became afebrile and the inflammatory markers normalized. After a one week follow-up with negative cultures and 3 consecutive dressing changes, the chest was closed and recovery uneventful. The patient was discharged to an outside hospital at six post-operative days.

**Results:** All the samples which were analysed histologically and for different pathologic stains as well as and by broad spectrum culture analysis, remained negative throughout. At one month follow-up the patient remained well and without fever or inflammatory response.

**Discussion:** Although several published case reports about tissue necrosis and anastomotic rupture following BioGlue use, the formation of a sterile abscess is a rare complication. In this patient, the peri-graft effusion might have been an allergy like inflammatory response to the bovine albumin within BioGlue. A prolonged postoperative mediastinal drainage when using BioGlue might prevent this rare complication.

O323

### LONG TERM EVALUATION OF A NOVEL TISSUE-ENGINEERED HEART VALVE WITHOUT ANY FOREIGN MATERIALS

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**Objectives:** We are developing a novel autologous tissue-engineered heart valve with a unique in-body tissue engineering. This is expected to be a viable bioprosthesis with better biocompatibility. In this study, we developed a conduit-type valve without any foreign materials and tested the feasibility and long-term availability in large animal experiments.

**Methods:** We created plastic molds for Biovalves with a 3D printer easily and quickly considering the recipient character. We embedded them in the subcutaneous spaces of adult goats for about 2 months. After extracting the molds with the tissue en-block and removing the plastic molds only, Biovalves with tri-leaflets similar to those of the native valves were constituted from completely autologous connective tissues and fibroblasts. Total 21 conduit-type Biovalves were implanted in the apico-aortic bypass or the pulmonary artery of goats, (8 and 13, respectively). No anticoagulants were used after implantation.

**Results:** The valves were successfully implanted and showed smooth movement of the leaflets with a little regurgitation in angiogram, and the maximum duration reached to 3 years 7 months. Histological examination of the Biovalves showed the autologous cells covering the laminar surface of the valve leaflets as the endothelium and also migrating into the leaflet body to construct characteristic tissues like native leaflets.

**Discussion:** The valves have a potential to be used for viable bioprosthetic valves and to keep better function and biocompatibility longer than current ones.

O324

### LASER FORMATION OF TISSUE-ENGINEERING STRUCTURES WITH ELECTRICALLY CONDUCTIVE FRAMEWORK

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**Objectives:** The aim of this work is the creation and research of properties of tissue-engineering structures by the method of laser structuring a nanocarbon framework in bioorganic matrices.

**Methods:** For the formation of the framework fibers from single-walled carbon nanotubes were used. Aqueous dispersions of nanocarbon fibers and proteins of albumin, collagen and amino sugar of chitosan were used as the initial medium for printing tissue-engineering structures. Printing of structures was carried out using the laser device generating pulsed laser radiation. The radiation wavelength was 1064 nm (100 ns pulse duration, 100 kHz frequency). Laser radiation using the scanner system of the device was moved on the dispersion layer. A computer model regulated the trajectory of the movement of radiation.

**Results:** We have demonstrated the effect of welding nanocarbon fibers into a framework under the action of laser irradiation. The functionalization of nanocarbon fibers by protein and amino sugar molecules were proven. It was found the diameter of nanocarbon fibers was increased by several tens of nanometers due to their wrapping with a bio-organic matrix. Tissue-engineering structures had a meshy frame. The mesh size was 100x100  $\mu\text{m}$ . The electrical conductivity of the structures increased several times and amounted to  $\sim 1 \text{ S/m}$  with laser irradiation. The hardness of tissue-engineering structures was 150-370 MPa. The cytological compatibility of tissue-engineering structures with fibroblast cells and endothelial cells was tested. The density of proliferating cells on tissue-engineering structures was 33% more compared to the control type. *In vivo* investigations indicated the rate of biodegradation of tissue-engineering structures when implanted in laboratory animals for 60-90 days.

**Discussion:** The investigated structures are promising for use in medical practice. Such structures can act as cardiac tissue-engineering implants since they have physicochemical and biological properties similar to the parameters of cardiac tissue.

### New Tools for Patients Follow-up in Renal Replacement Therapy

O331

### SAFETY ALGORITHMS BASED ON PATTERN RECOGNITION REDUCE FALSE POSITIVE ALARMS DURING HEMODIALYSIS

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**Objectives:** Alarm fatigue is a safety and quality problem where exposure to high rates of clinical alarms results in desensitization leading to cognitive bias or slowed response to alarms. One reason for alarm fatigue is false pressure alarms during extracorporeal blood therapies. It is clear that current machine algorithms based on simple threshold monitoring must be replaced by intelligent algorithms that avoid any false positive alarm.

**Methods:** Detailed analysis of arterial pressure, venous pressure, transmembrane pressure, ultrafiltration rate and relative blood volume during hemodialysis was performed using a patient data base. Main causes of pressure alarms were identified, analysed and replicated during simulated hemodialysis therapies with a FMC 4008 hemodialysis device and a patient simulator.

**Results:** Data analysis shows clear evidence that even slowly drifting adaptive baseline alarms which are currently in use lead to false positive alarms with a high probability during nearly every treatment. Pressure variations which are induced by local flow and blood viscosity changes can be identified reliably by use of algorithms based on pattern recognition. Pressure variations from patient movements and changes in blood pressure show a different pattern that can be used to distinguish between true alarm events and false positive events.

**Discussion:** For 25 years, it has been known that alarm systems which monitor whether a threshold value is exceeded have a rate of false alarms of 77-99,5% depending on the physiologic parameter monitored (Wiklund et al., 1994). New adaptive algorithms based on pattern recognition principles help to combine both maximum patient safety and avoidance of false positive pressure alarms.

**Acknowledgements:** The authors wish to thank Paul Camney who was the first to realize that pressure data of hemodialysis machines can serve as surrogate for patient condition during treatment.

O332

### INTRADIALYTIC ON-LINE MULTICOMPONENT TOTAL REMOVED SOLUTES MONITORING IN SPENT DIALYSATE BY A NOVEL MINIATURIZED OPTICAL SENSOR

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**Objectives:** The aim of this study was to evaluate intradialytic on-line multicomponent total removed solutes (TRS) monitoring in the spent dialysate, as potentially more objective parameter for removal of uremic solute markers urea, indoxyl sulfate (IS), and  $\beta$ 2-Microglobulin (B2M) compared to relative indices as removal ratio, using a novel miniaturized optical sensor during hemodialysis (HD) and hemodiafiltration (HDF) with different settings.

**Methods:** Ten ESKD patients (6 M and 4 F,  $60.2 \pm 16.8$  yrs) on chronic HDF were followed during 5 midweek dialysis sessions each (length 240min, HD: N=1, Qb=200ml/min, Qd=300ml/min, FX60; HDF: N=4, Qb $\geq$ 300ml/min, Qd $\geq$ 500ml/min, Vsubst $\geq$ 15l, FX800 and FX1000). Spent dialysate from the drain was monitored on-line by a miniaturized sensor prototype (Optofluid Technologies OÜ, Estonia). For total dialysate collection method as the reference, a spent dialysate sample from the tank at the end of each dialysis session was taken. The concentrations of urea and B2M were determined in the clinical laboratory. Concentration of IS was determined utilizing the HPLC. Based on differences between laboratory and optical sensor TRS values BIAS and standard error (SE) were calculated. A t-test was used to determine significant differences ( $P \leq 0.05$ ).

**Results:** The average TRS values of optical sensor and laboratory were  $510 \pm 86 \text{ mM/L}$  and  $485 \pm 106 \text{ mM/L}$  for urea,  $224 \pm 41 \text{ mg/L}$  and  $228 \pm 47 \text{ mg/L}$  for B2M, and  $732 \pm 371 \mu\text{M/L}$  and  $719 \pm 392 \mu\text{M/L}$  for IS, respectively. No statistical differences were found between optical and laboratory TRS values for any uremic solute. The relative BIAS $\pm$ SE values of the TRS were  $-7.99 \pm 19.43\%$  for urea,  $0.07 \pm 15.60\%$  for B2M, and  $-4.72 \pm 13.25\%$  for IS, respectively.

**Discussion:** Novel miniaturized optical sensor successfully carried out intradialytic on-line multicomponent TRS monitoring in the spent dialysate.

**Acknowledgements:** The study was funded by the EU through the ERDF H2020–SMEINST–2–2017, OLDIAS2–On-line Dialysis Sensor Phase2 project and the Estonian Ministry of Ed. and Res. under IUT 19-2.

O333

### PATIENT RELATED OPTIMIZATION OF FILTRATION LIMITS CARDIAC STRAIN DURING HEMODIALYSIS.

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**Objectives:** Hemodialysis (HD) is a lifesaving therapy in patients with end stage renal disease. Little is known about why life expectancy for these patients is reduced in Europe and USA as compared to Japan. The aim of this study was to evaluate the importance of the interdialytic weight gain (fluid retention in relation to estimated body weight in %, IDWG) in relation to the dialysis length (hours).

**Methods:** 20 hemodialysis patients performed besides their regular dialyses 1 hemodialysis/week within the frame of the study during a 3 week period. The baseline values and the change during HD of NT-pro-BNP (ProBNP) and Troponin T (TnT) at 180 minutes and pentraxin 3 (PTX) at 30 and 180min were compared with the change in IDWG divided by the hours the patient performed the HD as a marker of speed of ultrafiltration. The PROOF formula used was:  $[100 \times \text{Weight gain between dialysis (kg)}] / [\text{estimated body dry weight (kg)} \times \text{time of HD session (hours)}]$ . Spearman's correlation analysis was used to adjust for eventual outliers.

**Results:** There was a correlation between extent of removal of the IDWG/hours of HD: 1) the baseline values of ProBNP ( $\rho=0.54$ ,  $p<0.001$ ), TnT ( $\rho=0.37$ ,  $p=0.004$ ), PTX ( $\rho=0.26$ ,  $p=0.043$ ) and 2) the change during HD at 30 min of PTX ( $\rho=0.27$ ,  $p=0.038$ ) and at 180 minutes of ProBNP ( $\rho=0.68$ ,  $p<0.001$ ) and TnT ( $\rho=0.40$ ,  $p=0.002$ ). 3) a PROOF breakpoint  $< 0.63$  caused less release of ProBNP ( $p<0.001$ ).

**Discussion:** The increase in weight between dialyses should be related to the body weight. A larger IDWG gives worse conditions. An increased speed of fluid in relation to body weight removal causes a higher release of cardiac markers indicating a strain and damage to the heart by each HD in contrast to what would be expected. Patients should be advised to limit fluid intake in relation to their body weight and adjust the HD length.

**Acknowledgements:** Njurföreningen Norrland

O334

### DIALYSIS AND DIABETES: WHAT A MATHEMATICAL MODEL CAN TELL US?

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**Objectives:** Although diabetes is the leading cause of end-stage renal disease in many countries, data about its impact on patient-specific reaction to haemodialysis (HD) is scarce. Models that allow identifying and predicting patient-specific response to HD are in development and their improvement is encouraged by clinicians to obtain suitable decision support systems (DSS). Here, possible correlations between diabetes and

alterations in the patient-specific parameters identified by a multi-pool, multi-solute model, developed in our laboratory, are investigated.

**Methods:** Data acquired during the 'Dialysis' Interreg Project (141 patients, 4 centres, 1276 HD) have been used. For each patient, a set of specific parameters was identified: one modulates the permeability at the capillary wall ( $\rho$ ), the others account for altered transport of catabolites and electrolytes at the cellular wall ( $k_i$ ) and at the dialyser membrane ( $\eta_i$ ), where 'i' indicates different solutes. Patients have been classified as diabetic (D) or not (ND) and among Ds, as insulin-dependent (D\_ID) or not (D\_NID). Initial glucose concentrations and identified values of  $\rho$ ,  $k_i$ ,  $\eta_i$  in the different groups have been compared using proper statistical tests ( $p<0.01$ ).

**Results:** There were 37 (26,4%) D patients and 16 of them were D\_ID (43,2%). Initial plasma glucose concentration statistically differs both between D and ND and between D\_ID and D\_NID patients. Among the patient-specific parameters  $\rho$ , and  $K_{Na}$ ,  $K_{Mg}$ ,  $K_{Creatinine}$  and  $K_{Bicarbonate}$  results were different between D and ND. The values of  $\eta_{Na}$ ,  $\eta_{Mg}$ ,  $\eta_{Ca}$ ,  $\eta_{Urea}$  and  $\eta_{Creatinine}$ , instead, significantly differ between D\_ID and D\_NID.

**Discussion:** Diabetes-induced autonomic and peripheral neuropathies can justify the finding of altered capillary wall permeability in D; moreover the use of insulin, protein based, seems to impact on the mass transport through the dialyser membrane. The proposed approach allows adding a new tile to the studies on how the diabetic pathology affects the response to the HD treatment.

O335

### PENTRAXIN 3 MIGHT BE A BETTER BIOMARKER AS CARDIAC CONDITION MORE THAN INFLAMMATION IN SINGLE HEMODIALYSIS.

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**Objectives:** Cardiovascular diseases shorten life span of hemodialysis (HD) patients. Recently pentraxin 3 (PTX) was considered as a biomarker of cardiac function, as well as an inflammatory biomarker. The aim of this study was to investigate if PTX was a useful biomarker to estimate patients' cardiac condition more than as inflammatory biomarker by analysing plasma in PTX, CRP, NT-pro-BNP (Pro-BNP) and troponin T before and after HD.

**Methods:** Twenty patients on chronic HD were studied in a prospective cross-over study with three modes of HD with polysulfone dialyzers: (a) dry-stored (F8HPS, Fresenius) with a low blood level in the venous chamber (DL), (b) dry-stored with a blood level kept high (DH), and (c) a wet-stored dialyzer (Rexeed18L, Asahi Kasei Medical) with high level (WH). PTX, CRP, Pro-BNP and troponin T were assessed before and during 180 min of HD.

**Results:** The mean value of PTX at baseline was correlated to Pro-BNP ( $\rho=0.52$ ,  $p<0.001$ ) and troponin T ( $\rho=0.363$ ,  $p=0.005$ ), but there was no correlation to CRP. The mean value of PTX was significantly increased at 180 min (by 57%) compared to baseline data ( $p<0.001$ ). The baseline values of PTX correlated with the extent of rise of PTX ( $\rho=0.89$ ,  $p<0.001$ ), such as a higher baseline was related with more extensive increase. The mean difference value of PTX between baseline to 180 min of HD was correlated to that of Pro-BNP ( $\rho=0.31$ ,  $p=0.025$ ), while there was no correlation to that of troponin T nor CRP. PTX rose less while on WH than on DL (mean  $2.9 \pm 1.4$  vs  $3.8 \pm 2.8$ ,  $p=0.025$ ). There was no difference in PTX at baseline among these three settings.



**Discussion:** These data indicate that PTX is a more evident marker for the influence of the cardiac condition than an inflammatory marker during HD. Dialysis with WH causes least air contamination to the patient what may be a plausible reason for the difference.

O336

### ASSOCIATION BETWEEN REPERFUSION RENAL ALLOGRAFT BIOPSY FINDINGS AND EARLY TRANSPLANT FUNCTION

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**Objectives:** To determine the value of post-reperfusion kidney biopsy predicting renal function and the incidence rate of DGF in deceased donor renal transplant recipients within 1 year after the surgery.

**Methods:** We retrospectively included 461 patients who accepted the donation after the death of citizen at our center. According to the Remuzzi criteria, we compared the pathological biopsy and graft outcome between right and left donor kidneys from the same donor. Remuzzi score  $\leq 3$  was divided into low group, 4-6 middle group,  $> 6$  high group. The incidence of delayed graft function (DGF), acute rejection (AR) and renal function within 1 year after surgery was observed. A multivariate analysis was conducted on the risk factors of kidney function and DGF incidence.

**Results:** Of the 461 recipients, 458 recipients have good graft function and 3 grafts failed. The reperfusion kidney biopsy, the incidence of graft DGF and renal function at each time point after transplantation between the left and right kidneys from the same donor were same ( $p > 0.05$ ). 88.1% was in low group, 10.4% in middle group, and 1.5% in high group. The incidence of DGF and the eGFR in different time points after transplantation were different ( $p < 0.05$ ). Multivariate analysis revealed that last creatinine before acquisition and Remuzzi score were independent predictors of DGF after transplantation ( $p < 0.05$ ). Vascular scores were independent risk factors affecting renal function at 1 year after transplantation ( $p < 0.05$ ).

**Discussion:** The reperfusion renal allograft biopsy can reflect the donor's baseline data and predict early renal function after transplantation. Renal vascular disease may be an independent predictor of poor prognosis of the graft.

### Non-Rotary Cardiac Devices

O341

### MYTH VS. REALITY OF INTRA-AORTIC BALLOON COUNTER-PULSATION THERAPY PROLONGED USE: META ANALYSIS VERDICT

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**Objectives:** Intra-aortic Balloon Pump (IABP) is undoubtedly the most widely used mechanical circulatory support system for a range of severe heart failure indications. Average duration of IABP use is 1-3 days, however its longer-term use has not been adequately studied. The objective

of this work is to establish the extent to which the IABP duration has been used, and also to examine the clinical outcome pertaining to prolonged use of IABP, using meta-analysis of literature data.

**Methods:** We defined IABP prolonged use as 7 days or longer; a choice based on a duration that is greater than double the current perceived average use. We systematically reviewed the literature (Pubmed, Scopus, Web of Science) and identified 14 studies that have prolonged IABP use between 1968 - 2018. Patients indications included heart failure (refractory, congestive, end stage), ventricular arrhythmia, myocardial infarction and cardiogenic shock.

**Results:** A total number of 1707 patients of both genders received prolonged IABP use with an overall average of 22.6 days. The studied cohort is split into:

Group 1:  $n = 1683$  adults, mean age 57.9 years, range 19-84 years old, average use of 21.2 days. Survival rate in this group was 64.4%.

Group 2:  $n = 24$  infants and children, mean age 5.5 years, range 7 days-17.5 years old, average use 24 days. Overall mean survival rate to discharge was 62.5%. At a mean follow-up of 85 months, all 15 survivors were alive and well.

**Discussion:** Due to its ease of insertion and relative low cost, IABP presents the first port of call in a wide range of heart failure scenarios. This analysis indicates that, age does not seem to be a significant parameter that affect outcomes of IABP long-term use. Although, IABP is perceived to be only a short-term support, its prolonged use is evident with successful outcomes. Further trials of IABP long-term use for children and adults is warranted to further establish the range of indications IABP can be utilised as a long-term support.

O342

### HEMO- AND FLUID DYNAMIC INVESTIGATION OF A VALVELESS PULSATILE PUMP FOR THE TREATMENT OF HEART FAILURE WITH PRESERVED EJECTION FRACTION

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**Objectives:** Nearly half of all heart failure patients suffer from heart failure with preserved ejection fraction (HFpEF). With no effective treatment, a major clinical need is presented. The aim of this study was the hemo- and fluid dynamic investigation of a valveless pulsatile pump for HFpEF using in-vitro, ex-vivo, computational fluid dynamic (CFD), and 4D flow MRI imaging studies.

**Methods:** The pump is a pneumatically driven pulsatile pump with a single valveless cannula that is to be implanted through the LV apex and is operated synchronously with the left ventricle. A prototype of the proposed pump system was fabricated. The pump was tested in an in-vitro hybrid mock loop system to evaluate its hemodynamic effect in four HFpEF phenotypes. Further hemodynamic evaluation was conducted by implanting the pump in an ex-vivo isolated heart model. CFD was employed for flow field analysis of two different pump designs—a symmetric and an asymmetric configuration. The results were validated by 4D flow MRI imaging of the respective prototypes.

**Results:** In the in-vitro studies, the pump augmented cardiac output (CO) by 14 – 30% and reduced left atrial pressure (LAP) by 14 – 30%. Ex-vivo

studies showed the similar hemodynamic trends: augmented CO by  $20.0 \pm 9.7\%$ , and reduced LAP by  $57.5 \pm 14.4\%$ . Fluid dynamic simulations revealed good correlation with the respective 4D flow measurements. The asymmetric configuration disclosed superior fluid dynamic performance with 94.5% of the initial blood being exchanged after 3 beats.

**Discussion:** It was demonstrated that the valveless pulsatile device can improve hemodynamics in HFpEF patients by augmenting CO and reducing LAP. Appropriate washout of the pump chamber is achieved by a persisting vortex evolving within the asymmetric design throughout each pump cycle. By demonstrating the hemodynamic effects and promising fluid dynamic performance of the pump, feasibility of the system as a potential treatment option for HFpEF patients is supported.

O343

### A NOVEL EXTRACORPOREAL SUPPORT SYSTEM FOR ECLS AND ECMO APPLICATIONS

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**Objectives:** Extra Corporeal Membrane Oxygenation (ECMO) is used to treat acute respiratory and circulatory failure. Recent advancements have led to impressive improvements in treatment results. Now clinicians urge for an ever-earlier ECMO application and a broadening of the indications: Mobility demand → Every ECMO patient needs to be transported under severely restricted space conditions. Cost demand → With rising ECMO numbers the cost ratio plays an increasing role for healthcare economics.

**Methods:** A novel system uses a recombination of existing technologies to create a straightforward solution with a size and complexity reduction. The key principle is the sourcing of pump energy from the oxygen pressure, which is required to ventilate the oxygenator anyway. This modification allows a purely pneumatic operation and the usage of pulsatile pumps.

**Results:** A pneumatically powered dual chamber pulsatile pump has been developed which avoids suction and high shear rates with improved wash out behaviour for the entire ECC system. The innovative pump is driven completely pneumatically without a complex and expensive driving console. Furthermore, a novel oxygenator has been developed. Initial prototypes of pumps and oxygenators have been optimized during multiple design iterations and tested in terms hydraulic characteristics and gas exchange properties on the bench using water glycerol and porcine blood. The entire system has a reduced surface area and priming volume and shows low internal pressure loss, excellent wash out and gas exchange parameters. The bench test results have been confirmed within a chronic GLP in vivo study in large animals. The new device's size and complexity have been significantly reduced compared to the state of the art systems.

**Discussion:** The novel approach brings many advantages for the next generation ECMO/ECLS systems particularly in terms of function, mobility and cost-effectiveness.

O344

### DEVELOPMENT OF A MAGNETIC LEVITATION SYSTEM FOR AN OSCILLATING PLATE PUMP

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**Objectives:** The objective of this study is the development of a magnetic levitation system of a new type of blood pump. A fully magnetically levitated plate is oscillating in a trapezoidal shaped pump housing to create a relatively blood gentle flow. The oscillating movement of the plate is realized with a combination of a driven harmonic oscillator and a 1-degree-of-freedom magnetic levitation system.

**Methods:** Multiple design concepts utilizing different electromagnetic principles were 3D modelled with *Solid Works* and imported into *Ansys Maxwell 3D* to execute electromagnetic FEM simulations. Based on the results, the most suitable concept was selected by means of electromagnetic performance, stability, use of space and general feasibility. A PID-controller was implemented using *Matlab/Simulink*. The feasibility and stability of the concept was tested on a full-scale prototype.

**Results:** The prototype demonstrates the feasibility and stability of the concept. The stabilization of the plate can be realized with an electric current that is about 7 times lower than bearable for the electromagnetic coils of the first prototype, allowing further miniaturization of future prototypes.

**Discussion:** Though the general feasibility of the concept could be demonstrated, tests of the pumping performances are still pending to evaluate the suitability of the pump as an implantable blood pump.

**Acknowledgements:** This cooperative project was supported by a Scholarship Award by the International Society of Mechanical Circulatory Support.

O345

### POPULATION BASED ENGINEERING TO TREAT THE MAXIMUM NUMBER OF PATIENTS

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**Objectives:** Proper anatomical fitting of implants is crucial for a successful clinical outcome. However, every patient's anatomy is unique and there is a wide variety in the anatomical and morphological characteristics among individuals. Virtual fitting based on imaging data of a high number of patients has crucial benefits compared to conventional approaches during the design process.

**Methods:** Population based engineering is a method that enables a virtual implantation combined with iterative design optimization based on 3D anatomical models created from imaging data of a high cohort of patients. This approach was successfully used during the design process of a novel inflow cannula for a Ventricular Assist Device and a Total Artificial Heart for maximizing the number of treatable patients. The objective was to create a design that contains all components, but which at the same time works for a wide variety of different body types and sizes.

**Results:** The virtual studies have proven to give results that may not have been possible with conventional approaches. Compared with cadaver studies, this approach was a more accurate and economical way for determining the device fit and identifying areas for improvement. Clinical trials and in vivo studies of the devices have shown positive outcomes. Virtual fitting was able to reduce the risk of inflow obstruction, device-vessel misalignment, unexpected variabilities in the patient's anatomy and improper patient selection due to anatomical constraints.

**Discussion:** Population based engineering is a cost-effective solution for including a large number of patients and anatomy variations in the design process. Additionally, this approach can be used for regulatory submission, e.g. to determine and justify anatomical and morphological eligibility or exclusion criteria for proper patient selection and/or the

correct implant size. This is especially important in consideration of the future requirements of the Medical Device Regulation (MDR) for patient-specific implants.

O346

### REGULATORY APPROVAL OF ARTIFICIAL-INTELLIGENCE-BASED MEDICAL DEVICES: CRITICAL POINTS

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**Objectives:** Since the latest revision of the Medical Devices (MDs) Directive, the European regulatory framework considers stand-alone software as a MD per se, as confirmed by the MD Regulation. The recent surge of AI tools, which promise to be ubiquitous soon, poses problems relative to their certification as MDs. In view of the already granted market approval in Europe and the USA for some AI-based MDs, we aimed to investigate the main relevant issues of such MDs' regulation.

**Methods:** Unlike traditional SW as MDs, for which established standards already exist (e.g., IEC 62304, "Software life-cycle processes"), for AI tools there are very few specific standards. The definition of AI, or intelligence itself, is by no means universally accepted. Thus, we limited the scope of AI to Machine/Deep Learning (ML/DL).

**Results:** 1) ML/DL tools are data intensive, so that a critical issue is how to construct a representative set of input data. E.g., IEC 62304 recommends that the manufacturer include software system inputs and outputs, in the SW requirements: this is clearly too generic for a DL tool. 2) Guidelines for acceptable input data size should be drawn, for a given system/software architecture. Moreover, quality check of training data should always be executed, before SW training. Quality of ML/DL tools and of physical architecture is also relevant. 3) Generally, DL tools lack an explicit declarative knowledge representation, hence it is difficult or impossible to assess to "reasoning" behind the algorithmic decisions. "Explainable" AI should be the standard, in order to rule out fuzziness in machine clinical decision making and impossibility to pinpoint the problem(s), in view of the legal responsibility for MD use. It has been found that DL performance conflicts with explainability, thus priority setting will be crucial.

**Discussion:** The regulatory treatment of AI-based MDs needs improvements, in order to have safe and effective products, as well as wider societal acceptance.

## Poster Presentations

### Cardiac Modelling

P111

### NUMERICAL PREDICTION OF HIGH SHEAR RATE THROMBUS SITES IN VADS

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**Objectives:** Patients using ventricular assist devices (VADs) still suffer from adverse events such as pump malfunctions or thromboembolic events. This can be caused by thrombi that have formed inside the pump (pump thrombus). Therefore, there is a great need to prevent such adverse events through engineering measures in the early development stage of blood pumps. Currently, a numerical model to predict thrombus sites inside VADs is still missing and the risk can only be assessed with in

vitro experiments in the late development stage. A recently found model for thrombus formation at high shear rates that was derived from simple stenosis experiments promises great potential in the application in computational simulations (CFD) of VADs.

**Methods:** In this study, advanced high resolution URANS simulations of rotational blood pumps were conducted with the flow solver of StarCCM+ (Siemens) at an operating point of 5L/min at 75 mmHg. A  $k-\omega$  SST turbulence model and the sliding mesh method was applied. The existing model for predicting high shear thrombus formation was applied and compared with observations from explanted pumps. Based on these results a modification of that model is proposed that suggests a wall normal transport due to the change of shear rate in flow direction. This modification was realized in the CFD by applying a correlation between the computed direction of the pressure gradient and the flow direction.

**Results:** The application of the model shows that thrombus sites are overpredicted in rotational blood pumps when compared with experimental results or observations from explanted pumps. However, with a modification of the model which proposes that the influence is a wall normal transport due to a change in shear rate, a good agreement was found.

**Discussion:** Since there is little data available in the literature that shows the position of pump thrombi the validity of these models remains unclear and has to be experimentally evaluated further.

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### MECHANICALLY REDUCING REGIONAL LEFT VENTRICULAR WALL STRESS AND IMPROVING EJECTION FRACTION IN HEART FAILURE PATIENTS

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**Objectives:** Heart failure with reduced Ejection Fraction (HFrEF) is a progressive disease with a low 5-year survival of <50%, which affects 23 million people worldwide. It is characterized by adverse remodeling of the left ventricle (dilated cardiomyopathy) due to an increase in filling pressures and myocardial wall stress. Pharmacological treatment and cardiac resynchronisation therapy have proven beneficial for survival. For patients with end-stage heart failure, a heart transplant or Left Ventricular Assist Device can be considered. A shortage of donors, patient selection and major downsides such as invasiveness and driveline infections limit the use of these treatments. Research has shown a 13% decrease in mortality for every 5% increase in left ventricular ejection fraction. Therefore, we developed a smart memory alloy configuration in order to increase the ejection fraction and obtain an increase of 3,5% in a bench model. To cope with ongoing left ventricular dilatation and rise in wall stress, this should be combined with adjustable and measurable ventricular restraint therapy. Our first aim is to measure local wall stress during a full cardiac cycle. Next, we aim to develop a mathematical model of the left ventricle to characterize the left ventricle in HFrEF patients.

**Methods:** We will characterize in vivo wall stresses during the full cardiac cycle using Transesophageal Echocardiography and a left ventricular pressure catheter in 10 patients undergoing cardiac surgery for heart failure. With these parameters, we will develop a simplified mathematical model of the left ventricle and we will improve our bench model for experimental testing.

**Results:** This research will provide a characterization of the weakened left ventricular wall and the determination of optimal smart material properties and configuration of the cardiac assist device.

**Discussion:** With this information, a patient-specific HFREF treatment device will be developed combining active cardiac support and restraint therapy.

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### COMPARATIVE ANALYSIS OF THE THROMBOGENIC POTENTIAL OF THE HEARTMATE 3 AND HEARTWARE HVAD CARDIAC PUMPS: A “NUMERICAL RATIONALE” OF CLINICAL OUTCOMES

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**Objectives:** The HeartMate 3 (HM3, Abbott Laboratories, USA) and HeartWare HVAD (Medtronic Inc., USA) clinically revealed a significantly different thrombogenic profile. In detail, while the HM3 has abated incidence of pump thrombosis, it was not associated with reduced stroke incidence. We numerically characterized and compared prothrombotic flow characteristics of the two pumps to provide mechanistic insights into observed clinical outcomes.

**Methods:** Computational fluid dynamics (CFD) simulations were performed to evaluate flow fields associated with periodic speed modulation in the two pumps (the artificial pulse for the HM3 and the Lavare cycle for the HVAD). One-way coupling to a lumped parameter model of the cardiovascular system was employed to account for physiologic boundary conditions. Results were compared to baseline simulations with constant pressure head and rotor speed to comprehensively evaluate geometrical and speed modulation effects. Analysis of the thrombogenic potential was based on calculation of: i) a specific platelet activation marker, the Platelet Activity State (PAS), using time course of viscous and Reynolds stress computed on platelet trajectories probed using Lagrangian particle tracking, ii) scalar washout, iii) volumes of flow stagnation, and iv) wall shear stress patterns.

**Results:** The HM3 and HVAD showed different PAS distribution. Speed modulation was associated with increased sum of viscous and Reynolds stresses and PAS magnitude. Analysis of temporal and spatial gradients of the shear stress histories calculated on platelet trajectories, volumes of stagnation, and wall shear stresses allowed identification of geometrical and operating parameters that might contribute to pump thrombogenicity and pump washout

**Discussion:** We compared prothrombotic fluid dynamic features of the HM3 and HVAD under realistic dynamic operating conditions. Our results provide a “numerical rationale” for the observed clinical outcomes.

**Acknowledgements:** This work was financially supported by Fondazione Cariplo grant #2015-1044

P114

### EVALUATION OF THE EFFECTIVENESS OF A ROTARY BLOOD PUMP TO SUPPORT PATIENTS AFTER FOUNTAIN OPERATION UNDER DIFFERENT TOTAL CAVOPULMONARY CONNECTION

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**Objectives:** The Fontan procedure is the current standard and the best palliative treatment for most patients with a functional univentricular heart. During the Fontan procedure the caval veins are anastomosed to the pulmonary arteries. The resulting total cavopulmonary connection (TCPC) allows for the support of an acceptable level of pulmonary circulation. At the same time there is the high risk of cardiovascular system failure due to the increased load on a single ventricle. In order to increase patient survival and quality of life, the different TCPC connections aimed at improving blood hemodynamic seems to be a logical treatment option.

**Methods:** A CFD study was carried out for four types of TCPC (TCPC-1, TCPC-2, TCPC-3, TCPC-4) to determine the optimal connection from the point of view of hydraulic and power losses with and without the rotary blood pump (RBP) connected. The distribution of scalar shear stresses (SSS) in the volume was calculated, allowing for evaluation of the effect of the RBP on the blood at the operating point of 2.2 l/min and 11 mmHg of head pressure.

**Results:** The results showed that the TCPC-3 connection has the optimal geometry, as there is a decrease in hydraulic losses corresponding to 5 mmHg, with power losses of 0.055 W. Hydraulic losses and power losses for other types of TCPC are equal to 6 mmHg and 0.07 W for TCPC-2, 9 mmHg and 0.08 W for TCPC-4, 10 mmHg and 0.09 W for TCPC-1 respectively. The geometry optimization of the TCPC connection can significantly reduce the system losses, but only the integration of the RBP prevents these losses even for non-optimal TCPC.

**Discussion:** Although the use of a pump has significantly reduced losses, the presence of increased SSS can have a negative effect on blood. The obtained values of SSS will allow in the future the estimation of the degree of blood damage and, as a consequence, hemolysis level.

**Acknowledgements:** This study was supported by the grant from Russian Science Foundation No. 18-79-10008.

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### MATHEMATICAL MODEL OF THE FONTAN CIRCULATION WITH THE VENTRICULAR ASSIST DEVICE SUPPORT

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**Objectives:** The Fontan procedure is performed for univentricular correction of the heart in pediatric patients with tricuspid atresia. In the Fontan circulation, the veins connect directly to the pulmonary artery. However, as a result of the procedure the load on the single functional ventricle of the heart and veins increases. Eventually, patients need a heart transplantation. A possible solution to the circulation deficiencies of the Fontan patients could be the ventricular assist device (VAD). The VAD is implanted between veins and the pulmonary artery. The aim of this study is to develop a model of the Fontan circulation with VAD support.

**Methods:** We use mathematical models to reduce the time and resources spent on in vivo research. Nonlinear mathematical models represent sections of the cardiovascular system (CVS) in the form of blocks. Pressure variations in the CVS sections are described by a system of differential equations.

**Results:** Applying VAD to the Fontan patients exhibits the potential to increase the venous return of blood. According to the Frank-Starling law, the stroke volume increases with increasing venous return. VAD creates a necessary pressure differential between the venous and pulmonary sections of CVS.

**Discussion:** The interaction model of the Fontan circulation with VAD support demonstrates the normalization of the pressure and volume distribution in the CVS. This indicates that the method studied in this work may be a way to optimize the health condition of patients after Fontan procedure.

**Acknowledgements:** This study was supported by the grant from Russian Science Foundation No. 18-79-10008.

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#### EFFECT OF ATRIAL INFLOW CONDITIONS ON VENTRICULAR THROMBOSIS RISK DURING VENTRICULAR ASSIST DEVICE SUPPORT: A NUMERICAL SIMULATION

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**Objectives:** High prevalence of thrombi around the Left Ventricular Assist Device (LVAD) cannula has been proven by pathological studies. Thrombogenic potential (TP) inside of the left ventricle (LV) is evaluated based on shear stress history (SSH) and residence time (RT) of particles; however, the effect of the atrial inflow – including rotation and uneven flow distribution of the pulmonary veins – is ignored. In this study, the influence of the atrial inflow on the TP was investigated via Computational Fluid Dynamics (CFD) simulations.

**Methods:** In a patient-specific left ventricle (LV) model under full LVAD support, the flow fields were simulated with three different inflow conditions: with perpendicular velocity to the inflow (LAper, flow rate: 3.5l/min), with an additional rotational component at the inflow (LARot: 35rpm) and with asymmetric inflow conditions (LAasym: 60%/40% left/right). Platelet motion was simulated with a combination of laminar and the Lagrangian methods for 7s. The TP value was calculated based on the RT and SSH of particles ( $0 < TP < 1$ , with higher values corresponding to higher risk).

**Results:** The ventricular flow patterns were comparable for perpendicular and rotational inflows and different for asymmetric conditions. TP values were different for each of the atrial inflow conditions (LAasym: 0.37, LARot: 0.35, LAper: 0.4). The number of the particles with a SSH value more than 0.6 Pa·s (non-outlier range) increased for LAasym simulation (LAasym: 11%, LARot: 5%, LAper: 3%;  $p < 0.05$ ); however the particles that remain for more than 7s inside the LV were higher for LAper simulation (LAasym: 13%, LARot: 18%, LAper: 19%;  $p < 0.05$ ).

**Discussion:** CFD is an advanced tool for the evaluation of ventricular flow during LVAD support; however, neglecting the atrial inflow conditions could lead to an inaccurate prediction of flow parameters linked to ventricular and pump thrombosis. Reliable evaluation of ventricular blood flow, therefore, requires the consideration of realistic atrial inflow conditions.

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#### THE HEMIDIAPHRAGM INVERSION IMPACT ON THE VENTILATION PARAMETERS – VIRTUAL EXPERIMENTS ON AN ARTIFICIAL PATIENT

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**Objectives:** There have been discussions on effects of pleural effusion on the breathing muscles dynamics. In particular, hemidiaphragm inversion influence on the pleural pressure (PPL) and ventilation parameters has not been precisely determined. The aim of this study was to analyze changes in PPL and ventilation parameters in patients undergoing therapeutic thoracentesis (TT). Particular attention has been paid to inversion of the hemidiaphragm caused by large one-sided pleural effusion. The analysis was based on virtual experiments performed on an artificial cardio-respiratory patient (AP).

**Methods:** TT was simulated on AP, which consists of several cooperating models of the respiratory system mechanics, gas transport and exchange, and circulation. Three scenarios were considered: a) proper work of the diaphragm, b) flattening and fixation of the hemidiaphragm due to the large amount of fluid, c) paradoxical excursion of the inverted hemidiaphragm.

**Results:** Simulations showed that during progressive pleural fluid withdrawal significant changes in the course of PPL were observed, particularly in scenarios b and c. Paradoxical excursion of the inverted hemidiaphragm significantly influenced the alveolar oxygen partial pressure (PAO<sub>2</sub>) due to a kind of pendelluft: e.g., air flows out from the corresponding lung during inspiration and thus it flows to the lung in the hemithorax without pleural effusion.

**Discussion:** Flattening and inversion of the hemidiaphragm have an influence on several physiological factors of which PPL and PAO<sub>2</sub> seem to be the most important. Hence, TT may improve pulmonary system function particularly in patients with inverted hemidiaphragm.

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#### SIMULTANEOUS NONINVASIVE ESTIMATION OF CARDIAC OUTPUT AND PULSE WAVE VELOCITY USING SINGLE RECORDING OF PERIPHERAL PRESSURE WAVE

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**Objectives:** There are currently no available noninvasive methods that allow for the simultaneous and reliable measure of cardiac output (CO) and pulse wave velocity (PWV) in the arterial tree. Both of those indices are important biomarkers of cardiovascular status.

**Methods:** Radial pressure profiles were recorded using applanation tonometry in a group of healthy subjects (N=18) and hemodialysis patients (N=30). Recorded pulse waves were used to estimate patient-specific parameters of the mathematical model describing the blood flow in the system of 55 major arteries. Calibration of the model with patient data allowed to calculate patient-specific CO and PWV between any two points in the arterial tree. In all of the patients and healthy subjects we

performed additional measurements of PWV using ECG gated applanation tonometry at two arterial sites (SphygmoCor, AtCor Medical) and CO using bioimpedance cardiography (PhysioFlow, Manatec Biomedical).

**Results:** The model was able to reproduce all of the recorded pressure profiles with high accuracy (average relative error < 10%). Model-estimated PWV highly correlated with the one measured using SphygmoCor device ( $R = 0.75$ ,  $p$ -value < 0.001). Model-predicted CO also correlated with the one measured by the bioimpedance cardiography, but at a lower level ( $R = 0.52$ ,  $p$ -value < 0.001).

**Discussion:** Our study demonstrates that coupling a mathematical model with a single peripheral pressure profile has the potential to provide information about cardiovascular state that was previously only available when using more complicated methods.

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### GRID-INDUCED NUMERICAL ERRORS FOR SHEAR STRESSES AND ESSENTIAL FLOW VARIABLES IN A VENTRICULAR ASSIST DEVICE: CRUCIAL FOR BLOOD DAMAGE PREDICTION?

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**Objectives:** Adverse events due to flow-induced blood damage remain a serious problem for blood pumps as cardiac support systems. The numerical prediction of blood damage via computational fluid dynamics (CFD) is a helpful tool for the design and optimization of reliable pumps. Blood damage prediction models primarily are based on the acting shear stresses, which are calculated by solving the Navier-Stokes equations on computational grids. The purpose of this paper is to analyze the influence of the spatial discretization and the associated discretization error on the shear stress calculation in a blood pump in comparison to other important flow quantities like the pressure head of the pump.

**Methods:** CFD analysis using seven Unsteady Reynolds-Averaged Navier-Stokes (URANS) simulations were performed. Two simple stress calculation indicators were applied to estimate the influence of the discretization on the results using an approach to calculate numerical uncertainties, which indicates discretization errors.

**Results:** For the finest grid with 19 million elements, numerical uncertainties up to 20 % for shear stresses were determined, while the pressure heads show smaller uncertainties with a maximum of 4.8 %.

**Discussion:** No grid-independent solution for velocity gradient-dependent variables could be obtained on a grid size that is comparable to mesh sizes in state-of-the-art blood pump studies. It can be concluded that the grid size has a major influence on the shear stress calculation, and therefore the potential blood damage prediction, and that the quantification of this error should always be taken into account.

### Soft Tissue Engineering

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### INTERCONNECTED 3D POROUS POLY(E-CAPROLACTONE) MODIFIED WITH CHITOSAN GRAFTED PCL FOR DRUG DELIVERY PURPOSES

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**Objectives:** (Bio)chemical modification of implants with drug delivery systems can support the body's natural healing capabilities by imitation of smooth tissue transitions along bone-tendon conjunctions. Modified interconnected 3D porous poly( $\epsilon$ -caprolactone) could act as a scaffold material for these implants.

**Methods:** Cocontinuous blends of poly( $\epsilon$ -caprolactone) (PCL) and polyethylene oxide (PEO) were prepared by batch blending in an internal mixer. Annealing and leaching of the blends with water yielded the porous PCL scaffolds, which were then modified with chitosan (CS) grafted PCL (CS-g-PCL) via dip coating. Nanoparticle systems containing CS and tripolyphosphate (TPP) were obtained via ionotropic gelation. These CS-TPP nanoparticles (CSNPs) can be attached to the modified scaffolds via a Layer-by-Layer (LbL) deposition approach involving alginate (ALG).

**Results:** Interconnected 3D porous PCL scaffolds with different morphologies have been prepared. Modification with CS-g-PCL, alginate, alginate fluorescein amine (ALG-FA), CS-TPP and fluorescein isothiocyanate labelled nanoparticles (CS-FITC-TPP) in a LbL approach was carried out. The blank scaffolds were analyzed in various ways, including via scanning electron microscopy and mercury intrusion porosimetry. Modified versions of the scaffolds were analyzed accordingly and the fluorescently labeled species were investigated via confocal laser scanning microscopy. CSNPs were loaded with bovine serum albumin (BSA) and layered on the modified scaffolds. Release of BSA from the scaffolds was investigated by ultraviolet-visible spectroscopy at 37 °C in phosphate-buffered saline (PBS).

**Discussion:** Porous PCL scaffolds with different pore sizes and morphologies can be obtained via annealing and leaching of cocontinuous PCL/PEO blends. Surface modification with CS-g-PCL, alginate and CSNPs open up possibilities regarding graded implant fabrication.

**Acknowledgements:** The master thesis is part of the DFG Research Unit 2180 "Graded Implants for Tendon-Bone Junctions" and supported by DOW®.

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### TOWARDS BIOBANKING OF TISSUE-ENGINEERED AMNIOTIC MEMBRANE

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**Objectives:** Human amniotic membrane (AM) is employed in a broad field of applications as a wound dressing, corneal treatment, and scaffolding material. However, mechanical properties of native membranes vary depending on the zone of the placenta where the AM is obtained. For this reason, mimicking the AM through tissue engineering is a way to obtain a membrane with standardized properties. One technique to mimic the morphology of the extracellular matrix of AM is electrospinning. With electrospinning, a nanometric arranged similarly to the AM extracellular matrix made of different polymeric blends could be obtained. In addition, the correct storage is a key point in order to guarantee the availability of such tissue-engineered constructs for clinical applications.

**Methods:** The AM extract is first prepared from native material and then freeze-dried. Afterwards, the AM-based scaffolds are obtained using blend electrospinning of polyethylene oxide and freeze-dried AM. Structural and compositional properties are assessed by scanning electron microscopy and confocal Raman spectroscopy. The mechanical

properties are evaluated by static mechanical testing before and after cryopreservation. The scaffold is then seeded with multipotent stromal cells. The biocompatibility of the tissue-engineered AM is analyzed using cytotoxicity assay.

**Results:** The tissue-engineered membrane presents a nanostructured morphology similar to the extracellular matrix of AM. The compositional results can detect the presence of collagen, fibronectin and hyaluronic acid in the final material. Preliminary results present changes in the mechanical properties related to the cryopreservation. The fabrication technique for the tissue-engineered AM seemed not to alter the biocompatibility with stem cells.

**Discussion:** The AM is ideal for treatment of different tissues, and in combination with polymeric materials by electrospinning, it could be a suitable candidate for a tissue-engineered membrane with standardized properties.

**Acknowledgements:** This work was supported by the German Academic Exchange Service (DAAD).

P123

#### INVESTIGATION OF BIOCOMPATIBILITY OF THREE-DIMENSIONAL CELLULAR AND TISSUE ENGINEERING STRUCTURES OBTAINED BY THE METHOD OF LASER PRINTING

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**Objectives:** The purpose of this work is the study of biocompatibility for samples of three-dimensional cellular and tissue engineering constructions for layer-by-layer repair of tissues of heart and blood vessels. Samples components are albumin, collagen and chitosan as matrices and carbon nanotubes as reinforcement. Three-dimensional structures are formed in layers by laser printing.

**Methods:** The biocompatibility of the samples and layers of their components was evaluated after their incubation with endothelial cells for 72 hours. Cell morphology was assessed by fluorescence microscopy and quantitative analysis was performed using MTT-test and real-time cell analysis by means of electrical impedance monitoring. For in vivo studies, samples were implanted in breasts of laboratory birds.

**Results:** It has been established that the layer consisting of albumin and nanotubes supports proliferation of cells the most. Moreover, all layers have surface properties that provide cell adhesion. Real-time cell analysis showed improved cell proliferation compared to control during the entire incubation time, however the greatest differences were observed after 25 hours of incubation. The morphology of the cells on the samples corresponds to the morphology of the control cells. With implantation of samples in experimental animals, the absence of their toxicity was observed.

**Discussion:** Samples of three-dimensional cellular and tissue-engineering structures are biocompatible because of characteristics of their components and suitable surface structure. The obtained samples can be used to repair damages in cardiovascular system. Due to the method of laser printing, samples can be made in any form, taking into account the features of the damaged area of tissues.

**Acknowledgements:** This work was supported by the project of the Ministry of Science and Higher Education of the Russian Federation (14.578.21.0234 RFMEFI57817X0234).

P124

#### SYNTHESIS OF COLAGEN BIOPOLYMERIC SCAFFOLDS MODIFIED BY CROSSLINKING, CHONDROITIN SULPHATE AND CARBON NANOTUBES FOR BONE REGENERATION.

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**Objectives:** Bone loss at implantation sites on oral cavities is a major problem for dental surgeons; in order to combat this issue, we developed 3 types of collagen biomaterial blends: chondroitin sulfate, carbon nanotubes, and electric stimulated.

**Methods:** Bovine collagen type I was dissolved at 4% in formic acid 0,1M (Synth - Brazil) and divided in 4 groups: A- with Chondroitin Sulphate (Sigma Aldrich); B - carbon nanotubes suspension (Sigma Aldrich); C - electric field; D - control group. All of the samples were crosslinked with NHS (N-hydroxysuccinimide esters) (Thermo Scientific-USA) and freeze dried at a LH2000 equipment (Terroni - Brazil). The samples were analyzed by: SEM; EDS; XPS; Bartha respirometry and FET.

**Results:** All of the samples have the same macroscopic morphology. The SEM of the group submitted to electric field shows organization of the collagen fibers. The EDS shows atomic content of carbon, oxygen, and nitrogen with other substances <1%. The XPS analysis suggests chemical modification of the collagen amine groups by the NHS and the Bartha respirometric method shows increase in degradation from 42 to 117 days. The FET test shows good tolerance of the material at the amount used for therapy in humans.

**Discussion:** The absence of contaminants within the samples and the increase in stiffness exhibit the compatibility of this material for use in bone augmentation in implantology. The material is shown to be not toxic, however more tests should be conducted prior to human use.

**Acknowledgements:** Acknowledgments to FAPESP for the grant PIPE 2 (17/50413-0).

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#### EFFECT OF GLUCOSE CONCENTRATION ON METABOLISM AND VIABILITY OF ENDOTHELIAL CELLS AND SMOOTH MUSCLE CELLS CULTURED ON THE SEMIPERMEABLE MEMBRANES

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**Objectives:** The aim of the study was to analyze the effect of glucose concentration in the culture medium on metabolism and viability of the human umbilical vein endothelial cells (HUVEC) co-cultured with the human umbilical artery smooth muscle cells (HUASMC) on polysulfone semipermeable flat membranes.

**Methods:** Cells were isolated from umbilical veins and arteries obtained by Caesarean section. Membranes with a surface area of 2 cm<sup>2</sup> were covered with fibronectin to promote cells' attachment and then were placed in specially designed inserts. Cultures were carried out in 12-well plates filled with culture medium containing glucose in normal (5 mM) or high (20 mM) concentration. The HUVEC and HUASMC were seeded on separate membranes or on both sides of the same membrane with density of 4,600 cells/cm<sup>2</sup>. Cultures have been conducted in standard

conditions (37°C, 5% CO<sub>2</sub>) for 7 days. Activity of the reactive oxygen species (ROS) and cell viability were analyzed using the flow cytometry (with DCFDA test and Annexin V / 7AAD test, respectively). Microscopic visualization of cells was made after fixation in formalin using hematoxylin and eosin staining.

**Results:** In the HUVEC co-cultured with HUASMC the ROS activity was lower by 17% in the normal than in high glucose concentration. In normal glucose concentration the ROS activity of the HUVEC co-cultured with HUASMC was lower by 8% than in HUVEC cultured separately, whereas in high glucose concentration it was higher by 19%. Viability of HUVEC cultured separately in normal glucose concentration was higher by 4% than in high glucose concentration. Microscopic visualization of cells did not reveal any morphological differences.

**Discussion:** In terms of the ROS activity the obtained preliminary results indicate a beneficial effect of HUASMC on HUVEC co-cultured in normal glucose concentration. The opposite tendency was observed in high glucose concentration.

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#### EFFECT OF THE PASSAGE NUMBER AND GLUCOSE CONCENTRATION ON VIABILITY OF ENDOTHELIAL CELLS CULTURED IN VITRO

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**Objectives:** The aim of the study was to analyze the effect of the passage number and glucose concentration on viability of the human umbilical vein endothelial cells (HUVEC) cultured in the culture flasks.

**Methods:** The HUVEC were isolated from umbilical veins obtained by Caesarean section. Cells after the 2nd and 7th passage were seeded with density of 4,600 cells/cm<sup>2</sup>. Glucose concentration in the culture medium was kept constant at the normal (5 mM) or high (20 mM) level or was switched every 24 hours from normal to high level or vice versa for 7 or 14 days. The cell viability, mitochondrial membrane potential ( $\Delta\Psi_m$ ) and activity of the reactive oxygen species (ROS) were analyzed using the flow cytometry (with propidium iodide, JC1 and DCFDA test, respectively). The glucose uptake and lactate production was also monitored. Microscopic visualization of cells was made after fixation in formalin using hematoxylin and eosin staining.

**Results:** After 7 days of culturing in medium with normal, high and variable glucose concentration the cell viability was higher respectively by 5%, 4% and 4% in the HUVEC passaged 7 times than in those passaged 2 times. After 14 days the differences were higher and equal to 21%, 21% and 31%, respectively. After 7 days  $\Delta\Psi_m$  was higher 1.3, 3.2 and 2.6 times in HUVEC after 7th passage than those after 2nd one, which were cultured in medium with the normal, high and variable glucose level, respectively. The glucose uptake and the lactate production were higher after 14 days and similar for all passages. The ROS activity was lower after 14 days of culturing for all cultures. Microscopic visualization of cells did not show any morphological differences.

**Discussion:** The obtained results show that the properties and phenotype of HUVEC after the second passage are closer to the properties and phenotype of native cells. The HUVEC after the 7th passage are better adapted to in vitro conditions deviating from physiological conditions.

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#### OPTIMISATION OF FLUID DYNAMICS INSIDE MACROPOROUS CRYOGELS USING A MICRO-PIV SETUP FOR BIOARTIFICIAL LIVER APPLICATION

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**Objectives:** Bioartificial liver devices aim to replace the detoxification and metabolic functions of the liver in people with liver failure. Shear stress and local velocities created by flow inside porous scaffolds need to be optimised to improve cell viability and avoid inflammation, however there are no relevant studies on the topic to date. Here, fluid dynamics inside porous cryogels were characterised using a purpose-built micro-PIV system. The aims were to develop a micro-PIV setup to record the flow inside cryogel structure and to characterise the nature of flow inside the cryogel channels by video post-processing in order to determine inlet design.

**Methods:** HEMA-based cryogels were synthesised by cryogelation technique and the microstructure was analysed by SEM, confocal microscope and  $\mu$ CT. Open porosity, overall porosity and permeability were extrapolated from  $\mu$ CT. A  $\mu$ PIV set up was developed to visualise flow inside cryogels which was composed of a digital camera with a long-distance microscope objective, a pulsating LED light source, a pump and an optical chamber to host the cryogel. Glass beads of 10  $\mu$ m size were used as tracers to mimic red blood cells. Videos were analysed with PIVlab in Matlab which allowed the interpolation of local velocities.

**Results:** Cryogels possessed an open porosity, with pore size up to 100  $\mu$ m, as well as an interconnected network of pores, making it suitable for a perfused system. Fluid dynamics were optimized using a  $\mu$ PIV setup which allows for the visualisation of flow inside the cryogel channels.

**Discussion:** Videos showed that internal flow did not reorganize itself through the channels and was found to be laminar with  $Re < 1$  with low vorticity. This suggests that molecules and toxins of the blood in the centre of a channel would not interact with hepatocytes seeded on the cryogel pore walls. Hence, flow reorganization could be promoted through a layered bioreactor. A layered cryogel structure was introduced to improve flow characteristics and improve toxin/hepatocyte interaction.

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#### SILK FIBROIN-BASED ELECTROSPUN SCAFFOLDS FOR REGENERATIVE MEDICINE

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**Objectives:** One of the most pressing issues of regenerative medicine is the fabrication of constructs with defined parameters in order to create conditions for proper cell adhesion and proliferation that will be close to



native tissue conditions. An application utilizing the electrospinning method may potentially solve this problem. The main goal of this study was to fabricate electrospun scaffolds based on silk fibroin and to characterize their properties.

**Methods:** Silk fibroin scaffolds were obtained by electrospinning method. There were two types of scaffolds: 1) silk fibroin scaffolds, 2) silk fibroin scaffolds with addition of 30 per cent of gelatin by mass. Scaffold structure was investigated by scanning electron microscopy and scanning probe nanotomography. Cell adhesion and proliferation were investigated in mice fibroblasts 3T3 model. The regenerative potential of scaffolds was estimated in a model of Wistar rat full-thickness skin wound regeneration.

**Results:** The electrospun scaffolds have fibrous porous structure with an average fiber thickness 300-600 nm. Both types of scaffolds are biocompatible in vitro. Presence of gelatin in scaffold composition improves cell proliferation. Scaffolds promote skin wound regeneration and restore native skin structure. Histological analysis did not evolve inflammatory process.

**Discussion:** Electrospinning is a promising method to obtain fibrous biomimetic constructs with defined properties. The biocompatibility of produced constructs was shown in vitro and in vivo. Both types of scaffolds have a significant regenerative potential and are prospects for different fields of regenerative medicine.

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#### CREATION OF A BIO-ARTIFICIAL LIVER: WHAT THE OPTIMAL RATIO OF CELLS OUGHT TO BE IN CELL-ENGINEERING CONSTRUCTIONS

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**Objectives:** In order to determine the most effective ratio of liver cells (LC) and multipotent mesenchymal stromal cells of bone marrow (MSC BM) into implantable cell-engineering constructs (CECs) used for a correction of chronic liver failure (CLF).

**Methods:** In order to create liver CECs we employed a biopolymer implant - a composition of a heterogeneous collagen-containing gel, which concluded viable LC and MSC BM in ratios:1:1, 5:1 and 10:1 respectively. CECs with different ratios of LC and MSC BM were implanted into the livers of rats (n=40) in which CLF was modeled by using CCl<sub>4</sub>. The efficiency of the regulatory effects of CECs (with different cell ratios) on regenerative processes in livers were assessed by using biochemical, morphological and morphometric methods at different periods after their implantation.

**Results:** During studying of the liver CECs with various ratios of LC and MSC BM (1:1, 5:1, 10:1), it was found that the most optimal ratio of cells into the CECs was 5:1, because at such ratio of cells the most distinct normalization of morphological and functional liver parameters as well

as maintenance of the structural homeostasis within the CECs took place within 365 days after modeling CLF.

**Discussion:** The effective correction of CLF can be carried out by using the implanted liver CECs, in which donor liver cells and MSC BM are presented in ratios – 1:1, 5:1 and 10:1. But analysis of prolonged correction of liver morphological and functional parameters at CECs using allows to recommend the preference using of CECs using with ratio 5:1, because prolonged preservation of structural homeostasis into themselves CECs makes possible to prognosticate their prolonged regulatory action on the liver tissue at CLF, especially for recipients on a waiting list for liver transplantation.

P1210

#### GENERATION, CULTIVATION AND CHARACTERIZATION OF LARGE SCALE STEM CELL DERIVED BIOARTIFICIAL CARDIAC TISSUE

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**Objectives:** Generation of a large-scale stem cell derived bioartificial cardiac tissue (BCT) as a potential therapeutic option to replace damaged myocardium after myocardial infarction.

**Methods:** Large-scale BCT was generated from cardiomyocytes (CMs) derived from human induced pluripotent stem cells (hiPSC) mixed with human fibroblasts and a hydrogel solution in a newly designed tissue chamber with a diameter of 43 mm. The BCT development was microscopically recorded and evaluated. Maturation of CMs within the tissue was confirmed by immunofluorescence staining for cardiac markers ( $\alpha$ -SA, cTnT) as well as cell junction proteins (Con. 43, N-Cad). The integration of the tissue chamber in the novel custom-made bioreactor allowed investigating the initial parameters for electromechanical stimulation (such as pressure, stress amplitude, frequency) necessary for proper tissue maturation in a more physiological manner. To investigate the calcium handling through the tissue a new transgenic hiPSC line containing a genetically encoded calcium indicator (GCaMP6f) has been developed.

**Results:** Microscopically evaluation at different time points reveals a progressive reduction of nearly ~27% of the initial volume after 15 days, corresponding with an increased intercellular connectivity. After 48h, the tissue exhibited spontaneous contractions and coordinated and rhythmic contraction within the whole tissue after 6 days. As preliminary analysis, the beating of the tissue was video-optically recorded showing an estimated beating rate of 102 beats per minute. Immunofluorescence staining performed at day 15 showed highly organised cross-striations of sarcomere proteins. Initial integration of the tissue chamber in the bioreactor and implementation of the new GCaMP6f reporter cell line is still on-going.

**Discussion:** The initial results confirmed that is possible to generate a large-scale BCT. However, the properties of the tissue have to be further investigated.

#### Hemodialysis

P131

#### CONTRAST - INDUCED NEPHROPATHY, OLD STORY - NEW TWISTS

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**Objectives:** Radiological procedures utilizing intravascular iodinated contrast media are being widely applied for both diagnostic and therapeutic purposes and represent one of the main causes of contrast-induced nephropathy (CIN). In this hospital-based study we tried to assess predictors for the development of CIN in patients undergoing cardiac catheterization.

**Methods:** A total of 5604 patients undergoing coronary angiogram/PCI from 2007-2017 were enrolled in the study. Multivariate predictors of CIN were identified by logistic regression using stepwise selection with entry and exit criteria of  $p < 0.1$ . A two-sided 95% confidence interval (CI) was constructed around the point estimate of the odds (OR). A  $p$  value  $< 0.05$  was considered significant.

**Results:** CIN occurred in 6 (1%) patients. The mean age of patients suffering from CIN was higher than in the whole population ( $66.5 \pm 31.15$  vs.  $58.66 \pm 28.57$ ,  $p=0.03$ ). Characteristics of patients who developed CIN were: older age, diabetes, higher creatinine and lower EF. The incidence of CIN in patients with diabetes was higher and statistically significant (84% vs. 16%,  $p=0.01$ ). Emergency cases were at higher risk of developing CIN than elective patients (85% vs. 15%,  $p=0.001$ ), respectively. Diabetes, CKD and EF  $< 50\%$  were independent predictors of CIN (RR 2.4, 95% CI: 1.88 - 7.132,  $p=0.008$ ; RR 3.1, 95% CI: 2.17 - 6.682,  $p=0.003$ ; RR 1.6, 95% CI: 2.88 - 7.132,  $p=0.01$ , respectively).

**Discussion:** The development of contrast-induced nephropathy in patients who underwent angiography and PCI was mainly related to older age, diabetes, lower GFR and heart failure, but not contrast material exposure.

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#### POTASSIUM LEVEL, MALNUTRITION AND MORTALITY IN DIALYSIS PATIENTS

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**Objectives:** Obtaining normal serum potassium level is an important goal in maintenance hemodialysis patients. Hyperkalemia is known to be associated with mortality. In this study we aimed to access the relationship between pre-dialysis potassium level, nutritional status and survival in dialysis patients.

**Methods:** This study used annual cohort of hemodialysis patients with 36 months of follow up. To determine the impact of potassium level on mortality, patients were followed from first potassium measurement until death or a censoring event; hypokalemia was defined by potassium levels below 5.5 mmol/l and albumin levels below 35g/l was considered an index for undernourished. Time-dependent Cox proportional hazard modeling was used to estimate the association between potassium level and mortality.

**Results:** 199 patients were included in the study. Mean age was approximately 56 years, about 59% were men and 23% had end-stage renal disease caused by diabetes. Albumin below 35g/l was observed in 26 (13%) of the patients. In the follow up period 40 (20%) patients died, comprising 24 (32%) of the 74 hypokalemic and 16 (19%) of 82 hyperkalemic patients. The Kaplan-Meier survival rate was significantly longer in the hyperkalemic population ( $34.30 \pm 0.71$  vs  $31.06 \pm 1.16$ ,  $p=0.055$ ). Hypokalemia, when defined as serum potassium  $\leq 5.5$  mmol/l, was associated with all-cause mortality (hazards ratio (HR) 1.857, 95% CI 0.986-3.496,  $p = 0.051$ ). The significance was lost in the model after adjustment for albumin level. Only albumin level determined mortality ( $p=0.03$ ).

**Discussion:** Lower potassium level was associated with all-cause mortality, but only as a confounding effect of malnutrition in dialysis patients

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#### DETECTION OF HUMAN PARVOVIRUS B19 IN URINE OF KIDNEY TRANSPLANT PATIENTS WITH ANEMIA AND GRAFT DYSFUNCTION

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**Objectives:** Human Parvovirus B19 (HPV B19) infection is known to cause pure red cell aplasia (PRCA) in kidney transplant recipients. We investigated the diagnosis and prognosis of HPV B19 infection by urine detection in kidney transplant patients with anemia and graft dysfunction.

**Methods:** 1195 recipients (living donor/donation after citizen's death: 492/703) were performed kidney transplant in our center from January 2015 to January 2018. Thirty-six patients diagnosed with HPV B19 infection during follow-up (3.01%, 36/1195). Among those, fourteen HPV B19 infected patients underwent transplanted kidney biopsy, including 12 persons with PRCA and graft dysfunction and 2 recipients with donor-derived HPV B19 infection. We collected the urine samples for detection of HPV B19 DNA copies at the day of kidney biopsy. Meanwhile, the biopsy specimen were studied for immunohistochemical detection by using polyclonal mouse Anti-Parvovirus B19 antibody.

**Results:** The mean diagnosis time of fourteen HPV B19 infected patients was 44 days (7-300days) after transplantation. The reticulocyte percentage was 0.1-0.2% and the mean hemoglobin level was 67 g/L (51-87 g/L). Among 12 biopsies with PRCA and graft dysfunction, 6 were diagnosed with T cell-mediated rejection (IA-IB-IIA), 3 borderline rejection, 2 without rejection signs and 1 was diagnosed with thrombotic microangiopathy (TMA). The patient, who was diagnosed with TMA, had performed ABO-incompatible kidney transplantation. It displayed glomerular microthrombus and diffuse necrosis of renal tubules on renal biopsy. Despite the treatment of IVIG, the graft was ultimately lost. The immunohistochemical result of the TMA biopsy specimen was positive and the others were negative. The result of urine detection for HPV B19 virus DNA copies was  $>10^5$ /ml with TMA and the others were negative.

**Discussion:** If the urine test is positive for HPV B19 DNA, parvovirus-associated kidney injury should be suspected.

P134

#### SEVERE SECONDARY HYPERPARATHYROIDISM ASSOCIATED WITH A HIGH LEVEL OF BONE TURNOVER MARKERS IN END-STAGE RENAL DISEASE PATIENTS

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**Objectives:** To investigate the influence of bone turnover markers in end-stage renal disease patients for severe secondary hyperparathyroidism (SHPT).

**Methods:** 221 ESRD patients with SHPT were split into two groups according to the iPTH level: Mild SHPT group (M-group) (iPTH130 -600 pg/ml, n=86) and severe SHPT group (S-group) (iPTH  $> 1500$ pg/ml, n=53). Serum BTM such as beta-CrossLaps ( $\beta$ -CTX) procollagen type 1 N-terminal propeptide (P1NP), osteocalcin (OC) were detected.

**Results:** Compared to the M-group, the patients in S-group had higher levels of the  $\beta$ -CTX ( $5877.40 \pm 423.01$  pg/ml, vs  $3206.90 \pm 1438.27$ ),  $P=0.000$ , the P1NP ( $1145.50 \pm 171.36$  ug/ml, vs  $440.00 \pm 308.12$ ),  $P=0.000$ , the OC ( $258.52 \pm 48.61$  ng/ml, vs  $221.74 \pm 75.99$  ng/ml),

$P=0.000$ . ROC Curve analysis for the main risk factors of the parathyroid hyperplasia were  $\beta$ -CTX, P1NP, sALP, iPTH.

**Discussion:** Severe SHPT had higher levels of bone turnover markers, which may have aggravated CKD-MBD.

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### THE SHORT AND LONG TERM EFFECT OF METHYLPREDNISOLONE PULSE THERAPY FOR THE LUPUS NEPHRITIS TREATED WITH MUTI-TARGET THERAPY

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**Objectives:** Methylprednisolon pulse therapy was used in the initial phase of induction therapy in some patients. This study was conducted in order to examine the short and long term effect of methylprednisolone pulse therapy for the lupus nephritis treated with muti-target therapy.

**Methods:** The retrospective study included 43 patients diagnosed with lupus nephritis receiving muti-target therapy in our center, including prednisone combined with MMF and tacrolimus. 19 patients received methylprednisolon pulse therapy in the initial phase of induction therapy, 24 patients only received prednisone combined with MMF and tacrolimus. The dose of methylprednisolonpulse therapy was intravenous injection 500mg $\times$ 3 days and the following prednisone dose was 0.8-1.0mg/kg. We compared the primary and secondary outcomes of the two groups. In the patients combined with acute renal injury (AKI) subgroup, we also compared the primary and secondary outcomes between the two groups.

**Results:** In the primary outcome, the CR rates are similar between the two groups. We did not find significant difference in the total remission rate (either complete or partial) between the two groups ( $p=0.93$ ). The same is true for the remission time ( $p=0.87$ ). There was no significant difference in the time and number of patients entering hemodialysis between the two groups ( $p=0.65$ ). Although, in the AKI subgroup, there was a significant difference in the serum creatinine between the two groups in 1 month ( $P=0.045$ ). However, there was no significant difference in the following up, and the probability of achieving CR and replase (calculated by using the Kaplan – Meier method) also had no significant difference.

**Discussion:** Methylprednisolon pulse therapy seemed to have no significant effect on the long-term prognosis of lupus nephritis treated with muti-target therapy and it could not delay the patients' entry into hemodialysis. However, it may have had a better effect on the recovery of AKI patients and rapid relief of inflammatory reactions.

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### DEVELOPMENT AND EVALUATION OF THE EFFICIENCY OF AN ADAPTIVE SYSTEM FOR CONTROL TRANSMEMBRANE PRESSURE WEARABLE ARTIFICIAL KIDNEY

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**Objectives:** Solute movement from blood to dialysis solution is largely determined by transmembrane pressure (TMP). Accordingly, it was imperative to develop a TMP control system that allows for the maintenance of constant pressure. TMP in dialyzer is one of the tasks in wearable artificial

kidney development. In most of the known devices, transmembrane pressure is regulated only by adjusting the flow–pressure characteristics of the pump or mechanical uncontrolled clamping line.

**Methods:** A system was proposed which consists of a pinch valve, a DC motor and a control circuit. This device allows you to automatically adjust the pressure. At first, the device reads data from the differential sensor, which measures the TMP. After that, the position of the moving part of the pinch valve changes and the degree of clamping of the line changes, which in turn affects the value of TMP. The case of the device was made using a 3D printer. A test bench was created in order to determine the effectiveness of maintaining a constant TMP. It consists of five parts: two circuits with two peristaltic pumps, a dialyzer, a differential pressure sensor and a power source. The pressure from the primary circuit was varied by manually clamping the line and the degree of pressure differential compensation by the pinch valve was determined.

**Results:** The experiment showed that the TMP changes are fully compensated by the pinch valve. Accuracy of the developed device depends largely on accuracy of pressure measurement sensor. There was no change in the flow rate of the fluid, and this is important, because the rate of dialysate regeneration depends on the flow rate of the fluid.

**Discussion:** The experiment showed that this device can be used to control TMP. Accuracy of the system allows you to use it in the wearable artificial kidney.

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### COMPARATIVE EFFICACY AND SAFETY OF LOCK SOLUTIONS FOR THE PREVENTION OF CATHETER-RELATED COMPLICATIONS

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**Objectives:** To find the most appropriate lock solution for central venous catheters to prevent catheter-related complications.

**Methods:** Medline, Embase and Cochrane Central Register of Controlled Trials were systematically searched up to August 2018. We included randomized controlled trials comparing different lock solutions for adult dialysis patients ( $\geq 18$  years old). The trials should report at least one primary or secondary outcome. Network meta-analyses of outcomes were performed using the netmeta 0.9-6 package in R (version 3.5.1). Other analyses were carried out by means of the network and network graphs packages in Stata version 15.0.

**Results:** 50 trials (7142 patients) were included for this study. Compared with heparin 5000U/ml, antibiotic locks (antibiotics with TSC, EDTA, heparin 5000U/ml, low-dose heparin, or urokinase) and ethanol locks were more effective in preventing catheter-related bloodstream infections. Antimicrobial agents plus low-dose heparin (500-2500 U/ml), TSC and low-dose heparin locks had lower risk of bleeding events than heparin 5000 U/ml. No lock solution reduced rates of catheter malfunction and all-cause mortality compared with heparin 5000 U/ml. In summary, antibiotics plus low-dose heparin was ranked as the best lock solution. Considering the toxicity and drug resistance of antibiotics, ethanol could be the alternative antimicrobial lock solution. The overall results were not materially changed in sensitivity analyses.

**Discussion:** In total, according to the two-dimensional graph, antimicrobial agents plus low-dose heparin (500-2500 U/ml) was the highest ranking lock solution, as it reduced the rates of CRBSI and the risk of bleeding significantly. Ethanol was the promising alternative antimicrobial lock solution of antibiotics to combine with anticoagulant agents. TSC alone did not prevent CRBSI, but it is safe.

**Acknowledgements:** This work is supported by Special Funds for Scientific Research on Public Causes (201502010).

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### EFFECT OF PULSATILE AND NONPULSATILE BLOOD FLOW ON MEMBRANE FOULING DURING CONTINUOUS HEMOFILTRATION

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**Objectives:** Suppression of membrane fouling is an important key technology for the development of portable and implantable artificial kidneys. Among several factors known to affect membrane fouling, we focused on the blood flow condition. In the present study, to clarify the effect of pulsatile and non-pulsatile blood flow on membrane fouling, an ex vivo hemofiltration experiment using porcine blood was carried out.

**Methods:** Hemofiltration with filtrate recycling was performed under three conditions; pulsatile flow using a roller pump, non-pulsatile flow using a roller pump with three chambers to stabilize the flow, and non-pulsatile flow using a centrifugal pump. The blood flow rate (QB) was set at 50 or 100 mL/min and the maximum filtration rate at which irreversible membrane fouling would not occur was determined as follows; the first filtration rate (QF) was set at 2.5% of the QB for one hour (baseline), increased by 2.5% to 5% of the QB for one hour, and then returned to the baseline value for 20 min, and the transmembrane pressure (TMP) was measured to check if it had returned to the baseline value. This step was repeated until the QF reached 25% of the QB.

**Results:** For both blood flow rates (50 and 100 mL/min), the maximum filtration flow rate at which the TMP did not return to the baseline, implying that irreversible membrane fouling had occurred, was higher under the non-pulsatile flow conditions than under the pulsatile flow condition.

**Discussion:** The flow rate and the TMP changed periodically under the pulsatile flow condition and the peak TMP value was higher than that under the non-pulsatile flow condition, which increases the burden on the membrane. Thus, there is an increased likelihood of membrane fouling, as compared to the constant the pressure under the non-pulsatile flow condition.

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### EFFECTS OF ANTICOAGULATION METHODS ON BLOOD CLOT FORMATION IN THE ADSORPTION COLUMN DURING GRANULOCYTE AND MONOCYTE APHERESIS

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**Objectives:** Granulocyte and monocyte apheresis (GMA), in which granulocytes and monocytes are removed from the patient's whole blood, is used in the treatment of inflammatory bowel disease (IBD). The objective of the present study was to clarify the effects of the anticoagulation methods used on blood clot formation in the column during GMA.

**Methods:** Extracorporeal circulation was established for 60 minutes in male Sprague Dawley rats. A small column filled with 0.7 g of cellulose diacetate beads was used. We administered heparin at a total dose of

0.5 U/g by 2 different administration methods: the bolus administration method (single bolus administration prior to extracorporeal circulation) and the half bolus and half continuous administration method. During extracorporeal circulation the arterial pressure, column inlet pressure, venous pressure, and activated clotting time (ACT) were measured. The hemoglobin (Hb) concentration of the eluate of the residual clots was measured after the extracorporeal circulation.

**Results:** Extracorporeal circulation was performed stably for 60 minutes without events like in-circuit coagulation, in both groups. The ACT reached its peak value within 30 minutes in the bolus administration group, to decline gradually thereafter; on the other hand, in the half bolus and half continuous administration group, the ACT remained nearly constant during the extracorporeal circulation. The Hb concentration in the eluate was significantly lower in the bolus administration group than in the half bolus and half continuous administration group.

**Discussion:** The results indicated that even for the same total dose of heparin, clot formation in the column was influenced by the method of administration. We concluded that bolus administration of heparin, which led to early peaking of the ACT value was more effective to reduce residual blood clotting in the column as compared to half bolus and half continuous administration.

### Modelling and Analysis in Artificial Organs

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#### THE ASSEMBLY'S SHEATH FOR INDUCTIVE POWERING OF ARTIFICIAL ORGANS

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**Objectives:** The aim of this work is to design a reliable and efficient sheath for an implantable part of a device for the wireless inductive powering of artificial organs.

**Methods:** Wireless inductive power transfer can help get rid of the problems of recharge and wired constant power supply to artificial organs. However, the design of the implantable part of the transcutaneous powering device, or so-called assembly, is complicated by the lack of a comprehensive solution of technical and medical problems. The assembly must be encapsulated in the sheath which requires the choice of a bio-compatible and non-magnetic material. This eliminates the formation of eddy currents on the sheath, whereby negative influence on the energy transfer is minimized. At the same time, the material should have a low thermal conductivity (about 0.5 W / (m • K)) to prevent overheating of the tissue from the circuitry. The sheath of the assembly must have sufficient durability to protect the circuitry. The form-factor of the sheath should be minimized for the convenience of the patient (dimensions in the limit of 20 x 20 x 3 mm).

**Results:** The sheath was developed for the assembly prototype. The assembly provides an output power in the range 0.45...0.55 W for the axial distance between the coils in the range 10...20 mm and the lateral misalignment up to 20 mm. The calculation and measurement were performed in the air and with the samples of "skin-fat" biological objects, in order to determine and verify the sheath characteristics.

**Discussion:** We have developed and tested the assembly's sheath for inductive powering of artificial organs that meets technical and medical requirements.

**Acknowledgements:** This study was supported by the Ministry of Science and Higher Education of the Russian Federation (agreement № 14.579.21.0144, id RFMEFI57917X0144).

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**ACOUSTOMAGNETIC DETECTION OF MAGNETIC NANOPARTICLES IN A MODEL SAMPLE OF THE BIOLOGICAL SUBSTANCE.**

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**Objectives:** Magnetic nanoparticles (MNPs) are used in medicine for targeted drug delivery to the area of cancer. There is the problem of remotely determining in vivo concentrations of MNPs. The aim of our work was to experimentally test acoustomagnetic method (AMM) for detecting MNPs in a model sample of a biological substance (BS).

**Methods:** A colloidal solution of Fe<sub>3</sub>O<sub>4</sub> nanoparticles in a mixture of oleic acid and kerosene was used as a model sample. The weight concentration of the MNPs in the solution was 0.15%, and the viscosity of the solution was close to the blood viscosity. To detect MNPs in solution, a special experimental setup was developed. The installation consists of an ultrasound generator, a permanent magnet, a glass container with a solution, an induction multi-turn coil near the glass tube, and a voltmeter as a measure of the voltage across the coil. Ultrasound causes periodic movement of the particles of the solution together with the MNPs along the container axis. The magnet orients the MNPs in the direction perpendicular to the axis of the container. As a result, the summation of MNPs creates an alternating magnetic field with an ultrasonic frequency in the coil area. An alternating voltage (U) arises on the coil, as measured by a voltmeter.

**Results:** As measurements have shown, the magnitude of the voltage is proportional to the concentration of MNPs in the field of action of the ultrasound and the magnet field, as well as the intensity of the ultrasound. In particular, when the intensity of ultrasound was at the level of 0.02 W/cm<sup>2</sup>, U = 1mV was obtained.

**Discussion:** The experimental results are consistent with the calculated estimates and suggest that AMM can be used to detect MNPs in a real BS. Considering that the magnitude of the constant magnetic field in these experiments was 0.1T, we can also conclude that this method is safe, more accurate, and easier to implement than traditional X-ray and MRT methods.

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**MULTISCALE QUANTITATIVE ANALYSIS OF MICROSCOPIC IMAGES OF ICE CRYSTALS**

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**Objectives:** Modern research in cryobiology requires a deeper understanding of the influence of different factors on the cryopreservation of cells, tissues, and organs. One of these factors is ice crystallization which has a tremendous impact on the surveillance and quality of live objects during freezing, long term storage, and thawing. Analysis of this process requires software which should be able to obtain quantitative parameters of crystals in a human-like manner with acceptable processing speed. The purpose of this work is to consider the possibility of using the

multiscale image representation for the quantitative analysis of ice crystals.

**Methods:** In our research, we used microscopic images of ice crystals during crystals formation and thawing. In previous studies for the segmentation of ice crystals on the image, we used different approaches such as active contour. At the same time, it should be noticed that the speed of active contour expansion is low and thus time-consuming to process large time sequence. Thus, we suggest the application of a Gaussian Pyramid. This multiscale representation allows analysis at a low scale and improves at a high scale.

**Results:** We have analyzed multiple images using the proposed approach. The results in the first approximation show a 2-fold increase in speed when using our implementation of active contours. At the same time, the segmented areas of crystals correspond to the approach without the use of multi-scale image representation.

**Discussion:** The results of this work show that multiscale image representation can be applied to improve the speed and applicability of modern software for automated image analysis. The next steps will include applicability evaluation of multiscale representation for different cases as well as the development of software realizing vector processor architecture.

**Acknowledgements:** This work was supported by the DAAD Project Eastern Partnership (project number 54364768).

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**EVOLUTION-BASED PREDICTION OF ANTIBIOTIC RESISTANCE**

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**Objectives:** Biofilms on percutaneous leads of implanted blood pumps can cause severe infections. By treating infections with antibiotics over a long period of time, bacteria develop resistance. Today, in a clinical setting, antibiotic resistance is determined in-vitro for all available drugs. The aim of the EvolChip project is to predict the probabilities of resistances that might evolve and suggest combinations of antibiotics that slow down or even prevent future resistance.

**Methods:** The EvolChip reactor consists of an agar plate that serves as a diffusion and growth medium. Three membranes permit diffusion of active substances from microfluidic channels into the agar medium. Two of these channels serve as sources for different antibiotics, the third one as the sink. As a result, two overlapping drug gradients establish, with a low concentration near the sink and high concentration close to the sources. Antibiotics were mixed with a nutrient solution to promote bacteria migration towards higher concentrated areas. The bacteria contain a green fluorescent protein gene, which enables real-time tracking via polarisation filters and a camera.

**Results:** In preliminary experiments, the gradient course could be characterized by making use of fluorescent markers. Only resistant bacteria migrated towards the sources with high antibiotic concentration. The spatial distribution of bacteria facilitates the identification of optimal concentration ratios and mixtures of antibiotics. In addition, the speed of resistance evolution with respect to different antibiotic mixtures was determined.

**Discussion:** The proposed EvolChip concept showed promising results in preliminary experiments. Further geometries, growth media, and bacteria will be tested. In order to validate the method, patient strains with known treatment and resistance history will be compared to the predictions of the presented method.

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### QUALITY OF HEALTH CARE AND MORTALITY: THREE YEARS OF EXPERIENCE

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**Objectives:** The most fundamental goal in improving quality of care in hospitals is to eliminate unnecessary deaths. AIM: Three-year mortality and quality of care between 2015-2017 were evaluated in a tertiary nephrology clinic.

**Methods:** Audit of 392 deaths was carried out to reveal prevalence of suboptimal clinical observations. Admission pressure, direct admissions avoiding public healthcare system, severity of clinical conditions, elderly persons dying in the hospital, time of admissions and death were evaluated.

**Results:** In 2015 and 2016, 5.5 of every 100 inpatients died in the hospital, and this proportion decreased to 4.9 in 2017, with high percentage of patients who died in the first 24 or 48 hours from admission. The percentage of hospital deaths occurring in combination with AKI rose from 17% up to 37% over the three-year study period. Many patients who died with worsening CKD, were not ever referred to a nephrologist. More than half of patients were admitted on duty and died over weekends. About 5% of the hospital deaths were among patients aged 85 and over, and in 2017 that number rose up to 10%. Patients who died were seriously ill, at least 17% had malignancy. Most of the cardiovascular deaths were directly caused by ischemic stroke.

**Discussion:** In this study we did not find patients with preventable deaths. The results indicated that patients were seriously ill, elderly, some being admitted to hospital to die. Some of the patients were kept long period prior to death because of shortage on certain facilities. There is admission pressure and other external factors contributing to high burden in treatment of the dying patients. Still, further efforts are necessary for further providing and auditing health quality of care.

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### NON-TRANSMISSION SPECTROSCOPIC METHODS FOR NON-INVASIVE BLOOD GLUCOSE MEASUREMENT

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**Objectives:** Spectroscopic method is widely used for non-invasive blood glucose (BG) measurement. Despite the progress in implementation of transmission NIR-spectroscopic method, applicable mostly for earlap measurements, research of non-transmission methods allows for the expansion of spectroscopy range of use. The aim of research is to estimate the penetration depth for 1600 nm radiation using reflection NIR-spectroscopy. Sufficiency of penetration depth on this wavelength would allow for the use of a mathematical model implemented in the transmission method.

**Methods:** The developed experimental setup includes a semiconductor laser with wavelength of 1600 nm, two photodiodes, reflecting surface, control unit and power supply. For determining the position of optical elements a MATLAB program was developed, which calculated efficient detected radiation intensity depending on distances between the photodiode and the laser and between the laser and reflective surface. Scheme for measuring BG by reflection NIR-spectroscopy partially

repeats the scheme used for transmission method. The main differences are in location of photodiodes on the same plane with the radiation source, while their optical axes are co-aligned and parallel to each other, and the reflecting surface tightly abuts the back wall of analytical cell with test solution.

**Results:** The optimal distance between the photodiode and the laser is 5 mm, and between the laser and the reflective surface is 20 mm. This configuration allows intensity of the reflected radiation at about 20% of incident radiation to be obtained. Taking into account the permissible radiation density for skin, this value is enough for skin probing to a depth of 1.5 mm.

**Discussion:** Reflection NIR-spectroscopy is promising method for non-invasive BG measurement. Research of transmission method has shown that the penetration depth of 1.5 mm is enough for receiving information about BG. Thereby mathematical apparatus applied for transmission NIR-spectroscopy can be used and similar error less than 20% can be expected.

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### RESEARCH OF LAMINAR BOUNDARY LAYER INFLUENCE OF THE AIR FLOW ON THE MUCOUS MEMBRANE OF THE NASAL CAVITY

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**Objectives:** The purpose of the work is to develop a theoretical basis for determining the thickness of the laminar boundary layer in the nasal cavity during respiration.

**Methods:** Methods for obtaining basic scientific and practical results are based on the general principles of theoretical physics and aerodynamics in order to determine the laminar boundary layer of air flow in the upper respiratory tract.

**Results:** Using the aerodynamic approach to the study of respiratory and olfactory disorders revealed that the thickness of the laminar boundary layer decreases with decreasing equivalent diameter of the nasal cavity and with increasing Reynolds number, which characterizes the degree of turbulence in the air flow. Typical values of the thickness of the laminar boundary layer are in the range of 0.2-0.05 mm, depending on the mode of nasal breathing and the configuration of the nasal cavity. The data obtained characterizes the effect of airflow on the mucous membrane of the nasal cavity: if the heterogeneity of the mucosal surface extends beyond the boundary of the laminar boundary layer, then such portions of the nasal cavity are exposed to turbulent flow, which leads to overdriving. The size of the heterogeneities of the mucosa is determined by tomographic data during their processing at the sub-pixel level, which allows for the investigation of the effect of the intensity of the boundary elements of the walls of the nasal passages.

**Discussion:** A model of the distribution of airflow velocities in the nasal cavity in the turbulent mode is proposed; it has a logarithmic or power profile cross section, and within the laminar boundary layer the air velocity increases linearly to almost the max value. The current air velocity in the cross sections amounted to 5-7 m/s. The feature of the developed method is transitioned to hydraulic diameter, which takes into account complex configuration of the nasal cavity, as well as an anatomically narrow area - the olfactory slit.

**Acknowledgements:** We thank M.M. Kalashnik, the otorhinolaryngological department of KhNMU

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### MATHEMATICAL MODELING OF ACID-BASE BUFFER SYSTEMS IN HEMODIALYSIS PATIENTS

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**Objectives:** One of the objectives of hemodialysis (HD) is preserving optimal acid-base homeostasis. Bicarbonate is an obligatory component of dialysis fluids in order to titrate the acidic products of protein metabolism that accumulate between treatments. Even though bicarbonate can easily transfer across HD membranes, its plasma concentration does not equilibrate with that in dialysis fluid by the end of the treatment, for reasons unclear. We used a mathematical model to test whether an intradialytic increase in total non-bicarbonate buffer concentration in plasma (tNBBp) could explain this lack of equilibrium.

**Methods:** We modified the model of CO<sub>2</sub> and bicarbonate whole body transport proposed in 1995 by Rees and Andreassen, adding bicarbonate transfer from dialysis fluid to plasma via a dialyzer connected to an arteriovenous fistula. The model described the transport of O<sub>2</sub>, CO<sub>2</sub>, and base excess between different compartments: arterial and venous circulations, interstitial fluid and lung capillaries. In each compartment, equations describing the biochemistry of blood were solved to estimate the components of bicarbonate and non-bicarbonate buffer systems.

**Results:** The model qualitatively described the typical intradialytic pattern of bicarbonate concentration, pH, base excess, CO<sub>2</sub> and O<sub>2</sub> partial pressures observed in patients under routine thrice weekly HD. Model predictions were compared assuming a constant or increasing tNBBp during HD. When tNBBp was allowed to increase, the intradialytic increase in plasma bicarbonate concentration was blunted and the model predictions better fit published time-dependent intradialytic plasma bicarbonate concentrations.

**Discussion:** It was suggested in literature that HD treatment may stimulate an increased concentration of non-bicarbonate buffers and release of hydrogen ions from these organic acids. Our preliminary modeling results are consistent with this hypothesis and show that the model is capable of predicting the effect of the HD treatment on the buffer systems of the body.

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### CORRELATION BETWEEN DIFFERENT TYPES OF DE NOVO DONOR-SPECIFIC ANTIBODIES AND ANTIBODY-MEDIATED REJECTION AFTER RENAL TRANSPLANTATION

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**Objectives:** The main purpose of this study is to analyze the correlation between different types of dnDSA and AMR after renal transplantation.

**Methods:** We retrospectively analyzed the patients after renal transplantation from January 2002 to March 2017 in our Center. A total of 47 patients with positive PRA and confirmed as dnDSA were included, which were grouped according to the DSA binding to C1q, C3d and subtypes of IgG. Patients were divided into AMR and non-AMR groups according to the pathology of graft biopsy.

**Results:** The pre-transplantation dialysis time of the non-AMR group was longer than that of the AMR group in 47 patients with dnDSA positive

(35.4 +33.2 vs 9.8 +10.5, p=0.014). C1q-binding dnDSA had no significant effect on the graft survival after operation and biopsy. Among 47 patients with dnDSA positive, C3d-binding DSA group had lower graft survival time (p=0.009), higher HLA-DP mismatch ( $0.1 \pm 0.3$ , p=0.043) and higher percentage of pericapillary C4d deposition (p=0.042), with statistical significance. The graft survival rate of IgG3 subtype negative patients was higher than that of IgG3 subtype positive patients (p=0.003). Cox analysis found that the risk factors for graft survival included IgG3 (OR = 46.877, 95% CI = 4.211-521.830, P = 0.002), HLA-DR mismatch (OR = 0.103, 95% CI = 0.021-0.496, P = 0.005), proteinuria at biopsy (OR = 2.097, 95% CI = 1.184-3.713, p=0.024) and creatinine at biopsy (OR = 1.004, 95% CI = 1.001-1.007, P = 1.007)

**Discussion:** Single-center study showed that there was no significant correlation between the incidence of AMR and different types of DSA. The accurate HLA-DR typing should be emphasized during transplantation. The monitoring of specific types of dnDSA will help us to take interventions and thus contribute to the survival of transplanted kidneys.

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### TOWARD UNDERSTANDING THE IMPACT OF COCHLEAR MODEL CURVATURE, INSERTION SPEED AND IMPLANT STIFFNESS FOR THE PREDICTION OF INSERTION FORCES

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**Objectives:** To evaluate different designs or insertion techniques of cochlear implant electrode carriers (ECs) insertion forces are measured by a force sensor, which is mounted directly underneath an artificial cochlea model (aCM) leading to a summed force profile. One of the next steps in CI research leads from post-experimental evaluation of measured insertion force profiles to pre-experimental predictions of these profiles using analytical models based on an improved knowledge about factors impacting the insertion forces. Three likely factors were chosen for further investigation: speed, EC stiffness and curvature of the aCM.

**Methods:** Three aCM were fabricated out of PTFE blocks, each model having one constant curvature ( $r = 6.4 / 8.5 / 12.7$  mm). Additionally EC substitutes were fabricated using a two-component silicone, all with a constant diameter (0.7 mm), a total length of 20.5 mm and embedded bare copper wires. In order to vary the stiffness of the EC substitutes, one type had four and the other six wires embedded. They were inserted into the aCM with three different insertion speeds ( $v = 0.11 / 0.4 / 1.6$  mm/s). In order to increase reproducibility, insertions were conducted using an automated insertion test bench, comprising a linear actuator to clamp the EC substitutes and move them into the aCM and the force sensor underneath the model.

**Results:** In accordance with theoretical considerations all varied factors showed effects on the insertion force profiles. Increased insertion speed and sample stiffness increased the insertion forces, whereas an increased model radius decreased the insertion forces.

**Discussion:** The variance of measured forces within one set of parameters is due to the manual fabrication of samples and may be decreased by usage of commercial ECs. The influence of lubricants has to be analysed within future experiments.

**Acknowledgements:** The work was funded by the DFG within the Cluster of Excellence EXC 2177/1 "Hearing4all".

## Cardiac Devices - Monitoring and Testing

P211

### HOW CAN WE AVOID BIVENTRICULAR SUPPORT FOR CONTINUOUS-FLOW VAD IMPLANTATION?

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**Objectives:** Biventricular assist device (BVAD) is reported to be used in 3-5% of continuous-flow VAD (cf-VAD). Our recent strategy is to stabilize INTERMACS profile 1 or unstable profile 2 patients by paracorporeal VAD, central ECMO or IABP before proceeding to cf-VAD. Patients with RV failure are also aggressively treated by PDE-5 inhibitors or endothelin receptor antagonist at the outpatient clinic after implant. We examined validity of this strategy by analyzing BVAD necessity and patient survival. All cf-VAD was used for bridge to transplantation. Average waiting time for heart transplantation is nearly 3.5 years in our country.

**Methods:** 166 consecutive patients were implanted with cf-VAD. DCM was seen in 113, ICM in 14, dHCM in 12, post-myocarditis CM in 6, ARVC in 3, RCM in 3 and others in 15. 130 patients underwent primary cf-LVAD implantation. Pre-implant profiles of those patients were 2 in 62, 3 in 62 and 4 in 6. 36 patients were converted from another type of mechanical circulatory support (MCS). Actuarial survival was calculated by Kaplan-Meier analysis and comparisons were made using Log-rank method.

**Results:** No patient required RVAD at the time of implantation nor late after implantation. Average support duration was 787 days, and 53 patients were on the support longer than 3 years. During follow-up 56 patients reached transplantation, 9 weaned from VAD due to functional recovery and 80 on-going support. 1-, 2-, 3-year survival of 166 patients was 92.8%, 89.5% and 85.9%. There were no statistical differences between subgroups, such as sex, pre-implant profile, body size and primary/redo sternotomy.

**Discussion:** We successfully implanted cf-LVAD and managed in the outpatient clinic without RVAD support in 166 consecutive patients, including those who were severely ill in the pre-implant stage. Our strategy for stabilizing profile 1 and unstable profile 2 patients by other types of MCS and aggressive use of PDE-5 inhibitors or endothelin receptor antagonist seemed to be safe and effective.

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### BLOOD DILUTION AFFECTS HAEMOLYSIS: IMPLICATIONS FOR IN VITRO DEVICE TESTING

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**Objectives:** The American Society for Testing and Materials (ASTM) standards recommend bovine blood for in vitro testing of medical devices. Haemodilution is used to standardise haematocrit (HCT) to 30±2% with phosphate buffered saline (PBS). Research has shown that PBS increases red blood cell mechanical fragility and significantly increases haemolysis. Thus, the objective of this study was to investigate the impact of diluent and dilution for in vitro testing, which may result in future considerations for ASTM standards.

**Methods:** Bovine blood was diluted with either PBS or PBS+4g% BSA (bovine serum albumin) to a HCT of 30±2%. The blood was either pumped with the CentriMag<sup>®</sup> (Thoratec, USA) device under haemodynamic conditions or exposed to VAD-like shear for 6 hours. Blood was diluted to a range of 70-94% (n=40). Plasma free haemoglobin levels were measured to calculate the normalised index of haemolysis (NIH). Protein concentrations were measured.

**Results:** Haemodilution altered mechanical fragility depending on dilution and concentration. At a 70% blood dilution, PBS alone caused significantly higher haemolysis than PBS+4g% BSA. However, at a 90% blood dilution, PBS+4g% BSA caused significantly higher haemolysis than PBS alone. As such, a positive correlation was observed between dilution with PBS alone and NIH, whereas a negative correlation was observed between dilution with PBS+4g% BSA and NIH. Total protein concentration was significantly reduced at a 70% blood dilution with PBS alone but not at 90%.

**Discussion:** To reach a HCT of 30%±2%, this study recommends that bovine blood with a HCT of ≥37% should be diluted with PBS + 4g% BSA (>80% blood dilution), whereas blood with a HCT of <37% should be diluted with PBS alone (<80% blood dilution). Haemodilution with BSA maintains physiological protein concentrations and may reduce bias during haemolysis testing with high dilutions. Thus, ASTM standards could consider including BSA as a diluent, when needed.

**Acknowledgements:** Calon Cardio-Technology Ltd., Swansea University, Wales.

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### VENTRICULAR ASSIST DEVICE-INDUCED EXTRACELLULAR VESICLE PRODUCTION: A POTENTIAL BIOMARKER FOR VAD COMPLICATIONS?

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**Objectives:** Leukocyte activation is correlated to a reduced immune response in ventricular assist device (VAD) patients. Research has displayed leukocyte microparticle (MP; 200-1000nm diameter) increases following VAD shear stress. However, questions still arise regarding MP role, impact and biogenesis. MPs are plasma membrane derived, but there are smaller (50-100nm diameter), cytoplasmic vesicles (exosomes) which are often overlooked, or mistaken for MPs. As such, unless these vesicles are successfully separated, they should be categorised as extracellular vesicles (EVs). EVs are known to have roles in cellular communications and implications in diseases, and it is desirable to assess leukocyte sub-sets and any associated roles in VAD complications.

**Methods:** Bovine blood was diluted to a haematocrit of 30±2% according to ASTM standards. Blood was pumped for 6 hours in vitro with the CentriMag<sup>®</sup> centrifugal pump (Thoratec<sup>®</sup> Corp., USA). Flow cytometry determined a leukocyte EV population, having eliminated cellular debris. Platelet-poor plasma was tested for vesicle size and quantity using Nanoparticle Tracking Analysis (NTA), and immunoblotting conducted for exosome markers.

**Results:** Flow cytometry data displayed an increase in leukocyte death in the CentriMag, correlating to an increase in leukocyte derived EV production. Further analysis determined that these vesicles were mainly monocyte derived, suggesting a potential implication in inflammation. Preliminary NTA and immunoblotting displayed increased EVs following shear, namely exosomes.

**Discussion:** The presence of exosome marker staining and NTA size analysis displays evidence in favour of vesicle increase not being MP specific. As EVs have known implications in inflammation, infection and disease, these results suggest that exosomes may be associated with adverse events in clinical settings. Thus, exosomes should be further examined to elucidate any role as biomarkers of VAD related complications.

**Acknowledgements:** Calon Cardio-Technology Ltd. and Swansea University



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### LEFT VENTRICULAR ASSIST DEVICE THROMBOSIS: COMBINED APPROACH BY ECHOCARDIOGRAPHY AND LOGFILES REVIEW FOR DIAGNOSIS AND MANAGEMENT

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**Objectives:** Generally, LVAD thrombosis is diagnosed only by reviewing online logfiles that show an increase of power consumption and flow of the device. In order to increase diagnostic power and improve treatment management, trans-thoracic echocardiography was carried out to assess any blood flow changes.

**Methods:** Eight patients (M 7; F 1) experienced a total of 17 Heart Ware VAD thromboses. At echocardiogram, a 4-chamber view was obtained and the echo probe beam was aligned to blood flow of the inflow cannula. Continuous-wave Doppler was applied for the assessment of: 1) the increase of flow maximal velocity (Vmax) above basal ranges over several cardiac cycles and 2) the irregular discontinuous sound of the device. Online logfiles were reviewed for abnormal increase in power consumption and flow compared to basal data. All patients underwent thrombolysis with rTPA. Echocardiography results before and after treatment were compared.

**Results:** Thrombolysis was successful in all patients; one patient died due to cerebral haemorrhage. Echocardiograms before thrombolysis showed an increased mean Vmax of  $6.4 \pm 0.5$  m/sec, whereas after treatment Vmax was  $360 \pm 0.2$  m/sec ( $p < 0.001$ ). In all cases irregular, abnormal and discontinuous sounds were heard in thrombosed inflow cannulae. After thrombolysis only washing jet sounds were heard. Before thrombolysis logfiles showed a mean increase of power consumption of  $5.6 \pm 0.3$  watts, returning to basal values of  $3.8 \pm 0.3$  watts after thrombolysis ( $p < 0.001$ ). Before thrombolysis logfiles showed mean flow of  $6.2 \pm 0.6$  L/min, after treatment mean flow was back to normal ranges  $4.2 \pm 0.5$  L/sec ( $p < 0.001$ ).

**Discussion:** Transthoracic echocardiography showed a significant increase of blood flows in the inflow cannula of thrombosed LVAD devices, confirming findings of log files review. A combined approach by logfiles review and echocardiography is recommended in patients with LVAD thrombosis.

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### METHODS FOR TESTING THE FIRMWARE OF AN IMPLANTABLE HEART MONITOR

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**Objectives:** This paper describes the testing methods used in the development of software for an implantable heart monitor. It is a complex medical system. To identify errors made while writing software, as well as to detect deviations in the operation of the device in a timely manner, it is necessary to constantly carry out testing of all elements of the system.

**Methods:** Testing methods used in the development of an implantable heart monitor:

- debugging using JTAG,
- self-test system,
- testing via COM port,

- recording test information in the device's internal memory (data logging),
- interprocessor testing.

Consider some of them in more detail. Debugging and testing using JTAG: Due to the fact that the development was carried out in Code Composer environment, this allowed the use of a special script language GEL (General Extension Language). This is an expression language used by the CCS debugger. Launch GEL files are used to automate software development. GEL is an interpreted language, and its syntax is the same as C. Thereby it is possible to carry out tests, and change them, or add new ones in the process of the main software. Logging: One of the testing methods is to track changes in the state of the system over time. One way is to write data to the device's internal memory. But in such a way that you can track at what point in time the change occurred. For this you can use time stamps. If the data is recorded strictly with a certain frequency, you can do without time stamps.

**Results:** The presented test methods differ in that some of them can be used only in the early stages of development and some even when the device is at the end user.

**Discussion:** Cardiovascular diseases are very dangerous and difficult to diagnose. Early diagnosis and prediction of these diseases can lead to an improvement in the quality of life of patients.

**Acknowledgements:** This work was provided by the Ministry of Science and Higher Education of the Russian Federation (Agreement 14.575.21.0145, RFMEFI57517X0145 from September 26, 2017).

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### NOVEL CONTROL UNIT FOR THE ROTARY BLOOD PUMPS AIMED TO DECREASE LVAD MALFUNCTION

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**Objectives:** The left ventricular assist device (LVAD) consists of the pump, driveline and peripherals, all of which are potentially subject to failure. According to the recent research only 13% of failures were due to the pump itself, while over 60% were due to batteries, controllers and peripheral cables. Taking into account usability factors we present a novel control unit for the rotary blood pumps aimed to decrease LVAD malfunction.

**Methods:** The pump motor windings are leaded out by doubled wires (six total) via driveline to the extracorporeal splitter where the driveline is divided by two ways. Each way consists of three pump motor phase signals and three current sense signals from the other way. The two identical control units with integrated rechargeable batteries are connected to each way of the splitter simultaneously. This symmetrical peripherals architecture allows each control unit to drive the pump (in the active mode) or sense the parameters from the parallel way (in the passive mode). The control is based on the field oriented control method that allows the passive unit to continuously monitor the direction and magnitude of the magnetic field vector in the pump, which is created by the active unit and, upon loss of control, immediately begins to generate control signals with the necessary amplitude and phase. In case of mismatch of the parameters in any control units, alarms are to occur.

**Results:** Novel Sputnik LVAD was developed according to the described philosophy. This LVAD system has a backup feature which covers almost all peripherals including driveline, control system and battery. In case of the single fault condition in the peripherals the system continues to drive the pump with pause in the control less than 4 ms.

**Discussion:** Developed peripherals integrated in the LVAD allow reducing the risk of the device malfunction and improving usability.

**Acknowledgements:** The reported study was funded by RFBR and DFG according to the research project № 19-51-12005

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#### DEVELOPMENT OF ALGORITHMS AND METHODS FOR RECOGNITION HEART RHYTHM IN IMPLANTABLE CARDIAC MONITOR.

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**Objectives:** Continuous rhythm monitoring is useful for predicting, determining and treating a large class of diseases. An implantable heart monitor is useful for this purpose. It has small dimensions and records events over a timeframe measured in years. The objective of the study is to develop methods and algorithms for determining QRS-complexes and disorders in the work of the heart.

**Methods:** Several sources were used to obtain the ECG: large collections of recorded physiologic signals PhysioBank and BioModule BioHarness 3. Records of database have annotations with tags of QRS-complexes and conclusions of doctors. BioModule BioHarness 3 record lead V4 or V5 dependent on position electrodes. Sampling frequency is 1000 Hz. The records were made on the basis of I.M. Sechenov University Clinical Hospital No.1, Russia. The monitor of physiological parameters and the device for daily monitoring of ECG was installed by the patient simultaneously. Recording was carried out for 24 hours simultaneously on both instruments. The device for daily monitoring of ECG is used to determine the sensitivity and specificity of the developed methods. MATLAB was used to implement algorithms.

**Results:** Testing the algorithm with PhysioNet database resulted in a mean sensitivity of 99.21% and a mean specificity of 99.49%. Testing the algorithm with Biomodule 3 resulted in a mean sensitivity of 99.02% and a mean specificity of 93.69%. 16 records were analyzed. The analyzer gave an incorrect result in 2 cases.

**Discussion:** A careful study of records revealed that algorithm sets false tags in the absence of contact between the electrodes and the surface of the human body. The device for daily monitoring of ECG continues to operate normally at the same time. A careful study of the annotations PhysioNet showed that they contain errors. Some records have been shortened or excluded for this reason.

**Acknowledgements:** This work was provided by the Ministry of Science and Higher Education of the Russian Federation (Agreement 14.575.21.0145, RFMEFI57517X0145 from September 26, 2017).

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#### EFFICACY OF SECONDARY PREVENTION OF CRYPTOGENIC STROKE OR TIA IN PATIENTS WITH PFO SUCCESSFULLY TREATED BY PERCUTANEOUS APPROACH: 15 YEARS FOLLOW-UP

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**Objectives:** Cryptogenic stroke is the cause of 40% of ischaemic acute cerebrovascular events. Study aim was to evaluate the recurrence of ischaemic cerebrovascular events in patients successfully treated by percutaneous closure of patent foramen ovale (PFO).

**Methods:** From February 2004 to January 2019, 314 symptomatic (243 stroke, 71 TIA) patients, (153 M 161 F; mean age 41 yrs, range 10-69) underwent percutaneous closure of PFO. 151 patients/314 (48%) had concomitant migraine, 90 (60%) with aura. 7 different occluder devices were implanted by transesophageal echocardiography, for a total of 317 implants. During follow-up all patients underwent clinical (Rankin modified scale) and quality of life (SF36) evaluations, transcranial Doppler (TCD), trans-thoracic echocardiography, and MRI. Cerebral and angio-MR assessed the degree of lesions by quantitative and qualitative comparative analysis performed before and after treatment. Sizes of lesions were measured by manual segmentation on the axial, coronal and sagittal images acquired.

**Results:** Successful device deployment was achieved in 99% of patients; patients were discharged home within 3 days. Follow-up was 100% complete (median 55.4, range 1-178 months). At 6 months, Rankin scale was 0 ( $p < 0.0001$ ) in 230 patients (95%) affected by stroke and 10 patients reached score 1. Quality of life improved significantly ( $P < 0.0001$ ). In 101/151 patients (67%) with migraine, intensity and frequency of attacks significantly decreased ( $P < 0.0001$ ). TCD showed residual microembolic signals in 10 patients, 3 patients required secondary successful treatment for an associate defect. TTE (after 1, 3, 6, 12 months and once a year for 5 years) showed optimal sealing of all devices without signs of erosion, incomplete closure and thrombus. In 265 patients cerebral MRI showed no new lesions at 2 years.

**Discussion:** Our 15 year experience suggests that percutaneous treatment of PFO is safe and beneficial for secondary prevention of recurrence of acute cerebrovascular events irrespective of the device used.

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#### RESULTS IN PATIENTS WITH ENDOVASCULAR STENT GRAFT FOR ACUTE TRAUMATIC AORTIC RUPTURE: TWENTY YEAR FOLLOW-UP

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**Objectives:** Endovascular stent grafting is the standard treatment for patients with acute traumatic aortic rupture with extensive associated lesions. Very little long term information is available in large series.

**Methods:** From March 1999 to September 2018, 83 patients (72 M and 11 F; mean age:  $37.25 \pm 13.46$ ; range 16 to 69) admitted with acute or chronic traumatic aortic lesions underwent endovascular repair. 60 cases had acute traumatic aortic rupture, due to road accidents in 69 patients and accidental falls in 3 patients. All procedures were carried out in the angiography suite. Left subclavian artery was always identified. Patients were followed-up in the out-patient clinic and by yearly angio CT-scan with regard to survival and complications. The follow-up was 100% complete.

**Results:** Endovascular stent-graft treatment was successful in all cases of acute or chronic aortic injury. No post-operative paraplegia occurred. Control angiography showed optimal sealing and complete exclusion of the pseudo-aneurysm from blood flow with no primary endoleak. Patients underwent treatment of all associated lesions later on during hospital stay. Two patients died in the hospital: 1 patient of cerebral haemorrhage and 1 patient of sepsis. During the follow-up 5 patients died (survival: 91.4%) for causes unrelated to the aortic procedure. No cases of perigraft leakage or aortic disruption were detected. During

follow-up 1 patient had a steal syndrome and 1 patient paraplegia due to the covering of the left subclavian artery by endovascular graft. 4 years after treatment 1 patient had inner thrombosis of the graft developing a gradient; a new endovascular stent graft was deployed successfully. Freedom from complications was 92.3%.

**Discussion:** The outcomes over 20 years of follow-up proves that endovascular stent graft repair is the first choice treatment in patients with traumatic aortic injuries. Our experience demonstrates the feasibility and safety of endovascular treatment including patients with extensive associated injuries.

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#### INVESTIGATION OF THE ROLE OF PERICYTES IN THE IN VITRO MODEL OF BIOARTIFICIAL TISSUE FORMATION FROM hPSC-DERIVED CARDIOVASCULAR CELL TYPES

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**Objectives:** Cardiac grafts produced by combining human pluripotent stem cell (hPSC)-derivatives and tissue engineering are a promising therapeutic option for the replacement of cardiomyocytes (CM) lost due to myocardial infarction. Currently, the vascularization and maturation of such grafts are the most critical aspects. In addition to endothelial cells (EC), pericytes (PC) are known to play a crucial role in vessel development and stabilization.

**Methods:** Here, an hPSC-derived PC differentiation protocol is shown as well as an in vitro model for the investigation of the role of PCs in hPSC-derived bioartificial cardiac tissues (BCT).

**Results:** In the established differentiation protocol, the population giving rise to PCs (CD31-/PDGFRβ+) represented up to 94% of all cells and could be further purified via cell sorting. The obtained hPSC-PCs exhibited functional characteristics of PCs in co-culture assays, and showed similar gene expression profile to primary PCs. Differentiated hPSC-PCs were used w/ or w/o purified hPSC-ECs to generate BCTs and to address their effect on tissue morphology, metabolism, and electrophysiological parameters compared to control tissues containing primary fibroblasts (Fb). Interestingly, tissues with hPSC-PCs exhibited equivalent contraction forces compared to controls, but more organized sarcomere structures as well as improved longitudinal cell- and extracellular matrix orientation. Furthermore, the beating frequency and passive forces showed native-like values. Addition of hPSC-ECs resulted in spontaneously formed and maintained endothelial network structures, which were distributed throughout the BCTs and the involvement of PCs in such structures was demonstrated.

**Discussion:** The characterized and functional hPSC-PCs together with hPSC-derived CMs and ECs represent a promising cell source for myocardial tissue replacement therapy. Their dual ability in supporting EC function and matrix remodeling during tissue maturation makes them a favorable candidate to replace primary Fbs.

#### Hydrogels and Surface Modification

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#### EXAMINATION OF STEM CELL INTERACTIONS IN 3D POROUS HYDROGELS AS ARTIFICIAL BONE MARROW ANALOGUES

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**Objectives:** A great and long pursued goal in cancer research is the development of a suitable culture system for expanding haematopoietic stem cells (HSCs), which can thereafter be successfully used in transplantations. Since HSCs only reproduce in their natural environment, novel systems try to mimic the in vivo niche of HSCs, the bone marrow, in many factors such as material rigidity, chemical and cellular compositions. Mesenchymal stem/stromal cells (MSCs) are known to support HSC expansion. This effect is even greater when the cells are cocultured in a 3D environment. The aim of this project is to study the interactions of HSCs and MSCs in a conventional 2D culture compared with a 3D porous hydrogel, which was previously developed in our group.

**Methods:** The influence of HSCs on MSCs and vice versa will be studied on a transcriptomic level by next generation sequencing and verified by qPCR, cytokine arrays and western blots. To regain the cells from the system we want to make our hydrogel degradable by introducing disulfide bonds, which can be cleaved by reducing agents. Afterwards the RNA will be fixed within the cells and these will be separated by magnetic cell sorting, previous to RNA isolation.

**Results:** Previous experiments indicate that the 3D system has a positive effect on HSCs only in coculture with MSCs. With a deep insight into the transcriptomes of both cell types in coculture, we hope to identify key molecules playing a role in this phenomenon and in HSC expansion. Such key molecules shall further be tested for their influence on the coculture by inhibition or enhancement.

**Discussion:** The HSC expansion in vitro while maintaining their stem cell character has been a major and challenging goal in research for more than 60 years. By finding and influencing key molecules of the HSC-MSC interaction, we hope to further develop our 3D hydrogel system and increase HSC expansion in vitro.

**Acknowledgements:** The project is funded by the European Research Council (ERC).

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#### ROLE OF BIOPHYSICAL PARAMETERS IN TUMOR CELL DISSEMINATION TO BONE AND DEVELOPMENT OF A TUMOR CELL NICHE MODEL

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**Objectives:** Most breast and prostate cancers metastasize to the bone. To establish macrometastases, the disseminated tumor cells (DTC) need to have stem cell-like properties such as self-renewal and differentiation into other tumor cells. They compete with hematopoietic stem and progenitor cells (HSPC) and mesenchymal stem/stromal cells (MSC) for their specific microenvironment or 'niches'. Apart from biochemical factors in niches, biophysical cues also influence such interactions. The aim of this project is to investigate the intercellular crosstalk that occurs among these cells and the signalling pathways in a 3D porous hydrogel that was previously established in our group.

**Methods:** The hydrogel mimics in structure the trabecular region of the bone marrow. Cell-cell and cell-matrix interactions will be investigated using fluorescence imaging techniques. The pathways will be analyzed with the help of Western blots and specific inhibitors. Cytokine array and ELISA will be used for assessing auto- and paracrine communication via soluble factors. The biophysical parameters such as the 3D architecture and mechanical properties will also be tuned and their effect studied.

**Results:** The coculture of the breast cancer cell line MDA-MB-231 with bone marrow-derived MSCs and the leukemic cell line KG-1a reveal formation of spheroid-like structures in the gels. Experiments are being performed to study the cell-cell interactions within these structures.

Parallel experiments are being carried out regarding the effect of the 3D architecture, which will be introduced by varying the porosity of the gel.

**Discussion:** The results of these studies will help us to develop in vitro models of different tumor cell niches that will allow us in gaining a fundamental insight into the early events of bone metastasis. Tuning these parameters in the 3D bone marrow analog, we can have an understanding about how the biophysical cues affect the cellular activities.

**Acknowledgements:** We would like to thank the European Research Council for funding this project.

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#### FRICITION MEASUREMENTS ON CONTACT LENSES

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**Objectives:** The friction properties for the contact between contact lenses and the eyelid are the focus of current research in order to optimize the wearing comfort. The tribological system consisting of contact lens, eyelid and tear fluid should be considered. Suitable measurement conditions must be used for the examinations.

**Methods:** In this research the nano- and microtribological friction properties of hard and soft (hydrogel) contact lenses were investigated. The friction conditions were investigated by nano scratch test; moreover, a nanotribometer was used to evaluate the frictional properties during sliding on the lenses. Contact lens fluid was applied as lubricant for both cases.

**Results:** The friction coefficients of hard contact lenses and hydrogel lenses were detected for a range of speeds and loads. It was possible to examine the differences of the both contact lenses in detail. For the nano scratch and friction tests, there is a dependency of speed and normal force.

**Discussion:** In case of nano-scratch tests, the frictional properties were investigated while ploughing. This case shows the elastic- and plastic material properties besides the friction. In the case of the frictional tests, the measuring tip slides without ploughing. Due to the lubrication conditions, the soft hydrogel lenses feature a much lower friction coefficient. In case of the hard contact lenses, the coefficient of friction is comparable to the nano scratch test, as there is no lubrication film between both measuring tips and the contact lens. For further work it is mandatory to work under standardized measurement conditions.

**Acknowledgements:** We thank the company Wöhlk Contactlinsen GmbH (Kiel, Germany) and Anton Paar TriTec SA (Peseux, Switzerland) for supporting us with material and tests.

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#### HUMAN PLATELET LYSATE IN GELMA BASED HYDROGELS AS A 3D CELL CULTURE SYSTEM

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**Objectives:** For most in vitro cultivations of mammalian cells, culture medium is supplemented with serum, which contains important proteins and factors for the cell growth. Previous experiments showed already a positive effect of human platelet lysate (hPL) as a medium supplement for the two-dimensional (2D) cultivation of mesenchymal stem

cells (MSC). Since 3D culture systems gain big interest for in vitro applications and regenerative medicine, the influence of hPL on 3D cell culture models must also be investigated. Semi synthetic protein- based hydrogels represent a very promising 3D cell culture platform, which is biocompatible and biodegradable.

**Methods:** In our work, gelatine-methacryloyl (GelMA) hydrogels with a various degree of functionalization were dissolved in different hPL concentrations (0 %, 2.5 %, 50 %, 100 % hPL). The Influence of hPL on proliferation and differentiation of in GelMA encapsulated MSCs were monitored and measured via viability assays, immunocytochemical stainings and microscopy. Furthermore, the influence of hPL addition on mechanical characteristics of GelMA hydrogels was examined by swelling studies and rheometric analysis.

**Results:** The addition of hPL to GelMA hydrogels enhances the cell-spreading, the degree of differentiation as well as cell viability in concentration-dependent manner. Besides, the results of the rheometric tests showed an increase of hydrogel stiffness by using higher concentrations of hPL.

**Discussion:** Obtained results demonstrate that supplementation of hydrogels with hPL has a positive effect on cell growth and differentiation. Moreover, hPL can be a promising hydrogel stiffness enhancer for application in 3D bioprinting.

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#### HYDROPHOBIC MODIFICATION OF HYALURONIC ACID TO PREPARE A SHEAR-THINNING HYDROGEL FOR 3D-PRINTING

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**Objectives:** Developing new bioinks that are able to meet the crucial requirements for 3D bioprinting of different tissues has emerged recently as an important topic in the field of biomaterials. In this work, linear chains of hyaluronic acid (HA) were hydrophobically modified using hexadecyl short chains. The rheological properties as well as the 3D printability were investigated.

**Methods:** HA of  $M_w = 76.6$  kDa was hydrophobically modified by conjugation of parts of its primary hydroxyl groups with hexadecyl chains in two steps. The ratio of substitution was determined using <sup>1</sup>H NMR spectroscopy. Two different hydrogels were prepared at polymer concentration of 0.4 and 1.6 wt%. The rheological properties of both hydrogels were investigated using continuous flow experiment and cyclic strain time sweep experiment. Furthermore, the 3D printability of the hydrogels was studied using an extrusion-based 3D printer.

**Results:** <sup>1</sup>H NMR spectrum of the new product revealed that the substitution ratio was 40 mol%. Furthermore, the modified polymer was able to form stable hydrogels at both polymer concentrations whereas unmodified HA was unable to form hydrogel at the same concentrations. The rheological experiments showed decreasing of hydrogels viscosity by increasing the shearing rate which points toward a shear thinning property. Both hydrogels showed sharp decreases in the storage moduli upon applying high strains. The hydrogel prepared using 2.6 wt% of the modified polymer showed, however, higher shape-fidelity for 3D printing compared to hydrogel at polymer concentration of 0.4 wt%.

**Discussion:** Hydrophobic modification of HA using relatively short alkyl chains enables physical crosslinking between polymer chains to produce

biocompatible and 3D-printable hydrogel using low concentration of modified polymer. The rheological properties suggest their suitability as ink for extrusion-based 3D bioprinting

**Acknowledgements:** The authors would like to thank the European Research Council (ERC) for the financial support

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### VISUALIZATION OF HEMODYNAMICS: FABRICATION OF ARTIFICIAL ERYTHROCYTES WITH ENCAPSULATED PIV-PARTICLES VIA MEMBRANE EMULSION TECHNOLOGY SYSTEM

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**Objectives:** Visualization of hemodynamics often represents a simplification of blood as a monophasic fluid. In this study, blood is used as a multiphase fluid. The aim is to fabricate artificial blood to visualize hemodynamics with Particle Image Velocimetry (PIV). More specific, micro beads (ArtErys), which mimic the erythrocytes, are fabricated with a membrane-emulsion-technology system (MET). This system is developed and compared with the results (e.g. ArtEry size) of three other methods: microfluidic, electro-spraying and air flow.

**Methods:** The ArtErys are made of hydrogels (Agarose 0.3-0.7 wt.%) to match refractive index and biomechanics. For PIV tracking, particles (Sphericals®, 20 µm) are encapsulated directly into the ArtErys and are not added to the plasma where they might affect the hemodynamics. For the MET system, different oils (e.g. paraffin) are used as disperse phase. The Agarose/PIV mixture is pumped (50 to 100 ml/h) into a constant oil flow (5 to 40 ml/h) through a PTFE membrane (Reichert Chemietechnik GmbH, pore size 60 µm). The ArtErys are created during this process and transported away by the oil. The MET process is carried out in a heated water bath with a temperature of 55 °C.

**Results:** The ArtEry size depends mainly on two parameters: the flow rate of the agarose/PIV solution and the flow rate of the oil. A flow rate of 80 ml/h Agarose/PIV solution leads to smallest bead sizes with a mean diameter of 150 µm. High rates lead to merging of the ArtErys, which is reflected in a larger diameter. Furthermore, the bead size depends on the parameter oil flow rate. Here, a rate of 30 ml/h leads to the smallest beads.

**Discussion:** The MET method has been successfully implemented to fabricate ArtErys with encapsulated PIV particles. Compared to the other methods, the MET system fabricates the biggest ArtEry sizes (MET: 150 µm, microfluidic: 15 µm, electro-spraying: 80 µm, air flow: 100 µm).

**Acknowledgements:** The authors would like to thank our student G. Henschel for her constant support during the experiments.

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### NOVEL SURFACE COATINGS AS BIOCOMPATIBLE RESERVOIRS TO DEPLOY BMP-2 FOR BONE REGENERATION

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**Objectives:** This study was aimed at fabricating various layer-by-layer (LbL) systems using glycosaminoglycans (GAGs) with the capability to bind BMP-2 specifically in order to control osteogenic differentiation of cells by biocompatible release systems.

**Methods:** Heparin, chondroitin sulfate and their oxidized forms as polyanions were combined with chitosan and collagen I as polycations to form various multilayer coatings on model materials with the advantage of the intrinsic cross-linking formed between oxidized glycosaminoglycans (GAGs) and polycations, improving multilayer stability and affecting the release of BMP-2. The myoblast cell line C2C12, which can differentiate into osteoblasts was seeded on 5 µg/mL BMP-2 loaded multilayers. Cell viability, adhesion, osteogenic differentiation and BMP-2 release were investigated.

**Results:** C2C12 cells cultured directly on the top of multilayers showed that particularly BMP-2 loaded multilayers made of oxidized GAGs promoted an osteogenic differentiation that was nearly comparable to the positive control, when 5 µg/mL BMP-2 was added directly to the medium. Interestingly, the BMP-2 had synergistic effect on cell adhesion and spreading. BMP-2 in oxidized chondroitin sulfate multilayers was successfully loaded to the layers, sustainably released over time and affected cell differentiation more than the soluble BMP-2.

**Discussion:** The results show that oxidized GAGs forming intrinsically cross-linked multilayers are useful as reservoirs for sustained release of BMP-2 in which the intrinsic cross-linking affected BMP-2 release, improved multilayers stability due to the resulting stiff surface compared to the native ones, supported cell adhesion, proliferation and subsequent differentiation. This can pave the way for coating implants and scaffolds for repair and regeneration of bone fractures.

**Acknowledgements:** This work was supported by Bundesland Sachsen-Anhalt in the frame of Leistungszentrum Bio-und Systemtechnik and the International Graduate School AGRIPOLY.

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### POLYDOPAMINE-BASED COATINGS FOR A GRADED FUNCTIONALIZATION OF POLYCAPROLACTONE FIBER MATS

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**Objectives:** To develop an implant for the bone-tendon transition, it is our approach to optimize polycaprolactone (PCL) scaffolds by coating for a functionalization with drug release systems. The procedure during the functionalization should enable a release of growth factors in a spatially and temporally graded way.

**Methods:** The PCL fiber mats were prepared by electrospinning. Polydopamine (pDA)-based coatings were used to modify the surface of the fibers. To get thin polymeric, hydrophilic and biocompatible coatings we applied the pure pDA system and a co-deposition with amines (ethylenediamine (ED), hexamethylenediamine (HMD) and polyethyleneimine (PEI)). To functionalize our system with growth factors we used chitosan-based nanoparticles which can be loaded with the therapeutic proteins BMP-2 and TGF-β. By a defined dipping procedure we generate fiber mats with a graded functionalization.

**Results:** The measurement of the contact angle and the surface zeta potential showed a change from a hydrophobic PCL surface to a highly hydrophilic and amphoteric character for the coated systems. The

charged surface enables the further functionalization with loaded nanoparticles. The release of BMP2 and TGF- $\beta$  from the functionalized scaffolds was established. With a dipping procedure we are able to functionalize our scaffolds in a graded way. Confocal laser scanning microscopy with fluorescence-labeled NP showed that we could generate up to three differently functionalized regions.

**Discussion:** The results show the methodology is suited to prepare implants, which can release growth factors in a spatially and temporally controlled way. These graded implants can be used for in-situ tissue engineering.

**Acknowledgements:** The research project has been supported by the DFG in the framework of the Research Unit 2180 "Graded Implants for Tendon-Bone Junctions". We thank Robert Hänsch for CLSM measurements.

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### PREVENTING THROMBUS FORMATION ON ARTIFICIAL SURFACES BY APPLYING A HIGH FREQUENCY ALTERNATING CURRENT

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**Objectives:** Thrombus formation is a major risk of implants with artificial materials in blood contact. This adhesion of blood platelets plays an important role in thrombus formation and is influenced by the electrochemical conditions of the surface. The objective of this research is to change the electrochemical conditions by applying a low voltage, high frequency alternating electrical current to the surface, in order to avoid the adhesion of the platelets involved in thrombus formation.

**Methods:** Thin sheet samples with a thickness of 0.5 mm from different materials (titanium, stainless steel and copper) are attached to a high frequency generator and exposed to human whole blood under controlled flow conditions in an in vitro setting. An electrical current ranging from 50  $\mu$ A until 400  $\mu$ A in a frequency range from 10 kHz to 100 kHz was applied. The deposition of the blood platelets on the surface is identified and quantified by using fluorescent microscopy and digital image processing. The thrombus formation and therefore the anti-thrombogenic effect of the method is investigated in respect to different generator settings for each material.

**Results:** The characterization of all materials revealed changes of the surfaces in comparison to the zero samples. A differently pronounced reduction of thrombus formation with the proposed anti-thrombogenic method could be demonstrated on all three materials.

**Discussion:** Though the method could improve the anti-thrombogenicity under certain conditions, the mechanism is not fully understood yet and the parameters are not optimized. Further investigations will also focus on possible side effects caused by potential structural changes of blood proteins.

**Acknowledgements:** The work is funded by Volkswagen Foundation.

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### DIRECTIONAL AND CONVENTIONAL FREEZING OF CELL-SEEDED ELECTROSPUN FIBER MATS

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**Objectives:** Biobanking of in vitro engineered tissues presents obvious advantages associated with flexible preparation and shipping scheduling. To provide sufficient cryoprotection, alternative research models, freezing approaches and cryoprotective agents (CPAs) have to be established in view of complexity and diversity of composite tissues. Here, we evaluated the effects of conventional freezing (CF) and directional freezing (DF) for cryopreservation of cell-seeded electrospun fiber mats.

**Methods:** Fiber mats were produced from polycaprolactone and polylactic acid (PCL-PLA, ratio 200:100) using electrospinning. Structure of electrospun mats was characterized using SEM and FTIR, whereas their heat capacity was determined using DSC. The square-shape mats (1 $\times$ 1 cm, UV-sterilized) were seeded with HeLa cells (5 $\times$ 10<sup>4</sup>/scaffold) and cultivated at static conditions. Samples were frozen using 1 K/min to -80 °C (CF) or with the directional ice growth at the speed of 30  $\mu$ m/sec over a 2.6 mm gradient gap (from -4 to -10 °C) i.e. 4 K/min followed by further cooling to -20 °C at 1 K/min (translational cryostage) and to -80 °C with 1 K/min (LN flow cooling stage). The viability of cells was evaluated 24 h after thawing using fluorescein diacetate and propidium iodide staining.

**Results:** PCL-PLA mats exhibited no cytotoxicity on HeLa cells in vitro (viability higher than 90%). Only single cells survived both CF and DF when frozen in culture medium (negative control). In contrast, the majority of cells on fiber mats were alive after freezing under protection of DMSO.

**Discussion:** We showed that although not yet optimized, DF was as effective as CF for cryopreservation of cell-seeded PCL-PLA fiber mats. Preliminary studies on using antifreeze protein Type III (AFPIII) (10 and 100  $\mu$ M) as a sole CPA indicates that extracellular protection might not be enough to prevent freezing injury. Our recent work on electroporation of cells within electrospun fiber mats yielded encouraging results and the further initiative will be to deliver AFPs into cells using electroporation to provide DMSO-free cryopreservation of tissue-engineered constructs.

**Acknowledgements:** This work was sponsored by Short-Term Research Grant from Minerva Foundation as well as Ways to Research Program of the Leibniz University Hannover (60442522) and the Israel Science Foundation (grant number 930/16).

### Active and Composite Biomaterials

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### THE STUDY OF BIOLOGICAL LASER SOLDERS BASED ON BOVINE SERUM ALBUMIN AND SINGLE-WALL CARBON NANOTUBES USING DYNAMIC SCANNING CALORIMETRY METHOD

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**Objectives:** In this work, the denaturation behaviour of bovine serum albumin (BSA) in single wall carbon nanotubes (SWCNTs) aqueous nanodispersions during heating was analyzed using differential scanning calorimetry. Thermodynamic effects of BSA interaction with SWCNTs have been discussed. The calculation procedure for estimating the parameters of laser treatment on the basis of differential scanning calorimetry data is presented. Accurate calculation of the optimal parameters of laser treatment, under which there is complete denaturation of albumin and the solid nanocomposite material is forming, will minimize the thermal damage of the connected tissues, while not reducing the tensile strength of the seam.

**Methods:** Laser solders based on water dispersion of BSA (25 % w/w) and SWCNTs (0, 0.1, 0.01 and 0.001 % w/w) were used for differential scanning calorimetry study. Studies were conducted in hermetically sealed crucibles with heating rates of 3, 5, 10 and 20 °C/min.

**Results:** The specific energies and denaturation temperatures of the laser solders were measured. The use of SWCNTs led to a decrease in the specific energy of denaturation from 4.11 to 3.27 J/g and a denaturation temperature from 87.2 to 86.9 °C. The activation energy of albumin denaturation in laser solder has doubled during use SWCNTs

**Discussion:** Thermodynamic effects of BSA interaction with SWCNTs have been discussed, namely the changes in temperature characteristics of denaturation processes and the partial thermal destabilization of albumin. The energetic parameters of laser treatment have been estimated on the basis of differential scanning calorimetry data. The values obtained are optimal for complete BSA denaturation resulting in formation of solid nanocomposite material with minimal thermal damage of the connected tissues and sufficient tensile strength of the seam.

**Acknowledgements:** This work was supported by the project of the Ministry of Science and Higher Education of the Russian Federation (14.578.21.0234 RFMEFI57817X0234).

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#### ELECTRICAL CONDUCTIVITY OF LAYERS OF THE COMPOSITE BIONANOMATERIAL WITH SINGLE-WALLED CARBON NANOTUBES

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**Objectives:** Natural biological tissues have electrical conductivities and it is required that artificial tissues also possessed such a property. We investigated the specific conductivity ( $s$ ) of the bionanomaterial in the composition of bovine serum albumin (BSA) and single-walled carbon nanotubes (SWCNT).

**Methods:** We investigated the specific conductivity ( $s$ ) of the bionanomaterial in the composition of bovine serum albumin (BSA) and single-walled carbon nanotubes (SWCNT). The aqueous dispersion contained 25 wt.% BSA, 0.45 wt.% SWCNT. Dispersion on silicon substrates was applied by screen printing. After drying, the formed layers had approximate sizes: area – 5×20 mm<sup>2</sup>, thickness – 5÷15µm. For all samples the dependence of  $s$  on temperature  $t$  was recorded when they were in a thermostat. The  $s(t)$  curves were taken with a numerous cycle  $n = 1÷5$  with increasing and decreasing temperatures in the region of  $t \sim 20÷45$  °C.

**Results:** The following data was obtained. With increasing  $n$ , the values of  $s$  and the hysteresis on  $s(t)$  decreased: at  $n = 1 - s \sim 0.1÷1$  S/m, temperature resistance coefficient  $a \sim -0.004$  K<sup>-1</sup>, hysteresis on  $s(t)$ , i.e.  $R/R_0 \sim 0.90$ , where  $R_0$  is the initial resistance,  $R$  is the final resistance; at  $n = 5 - s \sim 3÷6$  S/m,  $a \sim -0.002$  K<sup>-1</sup>,  $R/R_0 \sim 0.95$ . Spectral studies in the optical range did not reveal any changes in the sample before and after a cyclic thermal action, which underlines the stability of BSA. Thus, in the layers of the BSA/SWCNT the  $s$  increases and the hysteresis on  $s(t)$  decreases with  $n = 1÷5$ . Apparently, this behavior is associated with an increase in the number of contacts between nanotubes in the albumin matrix.

**Discussion:** The studied layers of the composite bionanomaterial (BSA/SWCNT) has an acceptable value of specific conductivity ( $\sim 1÷10$  S/m) and mechanical properties (hardness  $\sim 300$  MPa) and is promising for biomedical applications.

**Acknowledgements:** This study was supported by the Russian Science Foundation, project No.18-79-10008.

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#### LAYERS OF THE COMPOSITE BIONANOMATERIAL AS THE STRAIN SENSOR

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**Objectives:** In medical practice, it is necessary to control the movements of various parts of the body: limbs, joints, chest, tumors, etc. For such purposes, the most suitable are the composite bionanomaterials in the composition of carbon nanotubes. We investigated the strain sensor (tensoresistor) based on the layers of the bionanomaterial contained biological material (bovine serum albumin, BSA - matrix) and multi-walled carbon nanotubes (MWCNT - filler).

**Methods:** The aqueous dispersion of 25 wt.% BSA/0.3 wt.% MWCNT was applied by screen printing on flexible polyethylene terephthalate substrates. After drying layers by laser irradiation ( $\sim 970$  nm) various parameters of layers were controlled, i.e. resistance  $R$ , bending angle  $q$ , number of cycles  $n$ , measurement time, etc. One measurement cycle corresponded to a change within the range  $q \pm 150$  °.

**Results:** The slopes of  $Sq = (1/R_0)dR/dq$  of the  $R(q)$  curves were considered to be strain-sensitivities, where  $R_0$  is the resistance of the sensor with  $q=0$ . It was found that with increasing the number  $n$ ,  $R$  and  $Sq$  increase and the hysteresis on  $R(q)$  decreases. For the tensoresistor obtained: specific resistances  $\sim 0.1÷1$  W×m,  $Sq \sim 1.0 \div 1.5\%/grad$ . These results are high.

**Discussion:** The physical picture of the change in the  $R(q)$  is as follows. When  $q>0$ , compression occurs, the amount of contacts between the nanotubes increases and the resistance drops relative to the state  $q=0$ . When  $q<0$ , the nanotubes move away from each other, the amount of contacts between the nanotubes decreases and the resistance increases relative to the state  $q=0$ . The examined layers of the bionanomaterial BSA/MWCNT as a strain sensor is of a particular interest for medical practice. In particular, for monitoring: movements (arms, blinking) and detection of signs of pathology (respiratory diseases, angina, et. al.).

**Acknowledgements:** The work was supported by the Ministry of Education and Science of the Russian Federation (Agreement 14.578.21.0234 RFMEFI57817X0234).

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#### POSSIBLE NON-INVASIVE CONTROL OF COMPOSITE BIONANOMATERIALS CONTAINING CARBON NANOTUBES

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**Objectives:** Recently, composite bionanomaterials containing biological materials and carbon nanotubes (CNTs) have been actively studied. CNTs may contain catalytic ferromagnetic nanoparticles and they can affect the properties of the composite bionanomaterial. To the study of this

issue, we paid attention. The object of the study was a composite nano-material consisting of bovine serum albumin (BSA - matrix) and single-walled CNT (SWCNT - filler).

**Methods:** Aqueous dispersion in the composition of 20 wt.% BSA and 0.01 wt.% SWCNTs were prepared using traditional nanotechnology methods (thorough mixing, ultrasonic homogenization, decanting, etc.). The optical density of dispersion was measured after a magnetic field gradient ( $\sim 1$  T/m) was applied to it for 100 hours. The obtained values were compared with the optical dispersion density in the absence of a magnetic field. Recent samples were considered control.

**Results:** It was found that under the action of a magnetic field, the optical density of the water dispersion of BSA/SWCNT in the region of 300 ÷ 800 nm decreases by  $\geq 10\%$  relative to control samples. Additionally, it was found that under the action of a magnetic field, the optical density of the aqueous dispersion of BSA/SWCNT decreases by  $\geq 10\%$  relative to control samples.

**Discussion:** The decrease in the optical dispersion density of BSA/SWCNT is caused by the deposition of nanotubes on the cell surface under the action of a magnetic field gradient. It is assumed that the single walled carbon nanotubes contain catalytic ferromagnetic nanoparticles. Estimates showed that in bionanomaterial at a level with concentrations  $\geq 0.0001$  wt.% CNT nanotubes can be fixed with modern sensors of a weak magnetic field with a threshold sensitivity of  $\leq 1$  pT. It was concluded that changes in implants in the BSA/SWCNT composite bionanomaterials can be observed non-invasively with the help of weak magnetic field sensors (for example, SQUID or CMFS).

**Acknowledgements:** This study was supported by the Russian Science Foundation, project No.18-79-10008.

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#### STATE-OF-THE-ART NEURONAL IMPLANT MATERIALS COMPARED WITH CNT-SILICONE RUBBER COMPOUND IN ANIMAL MODEL

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**Objectives:** Since Carbon Nanotubes (CNT) were shown to enhance neural outgrowth towards them, an approach with a CNT-Silicone rubber compound that immerses the CNTs and thus anchors them to the implant is investigated in an animal model

**Methods:** To compare the CNT-silicone rubber to state-of-the-art neuronal implant materials, 6 variations of the same implant with varying exposed materials were fabricated. The thin-film implants consisted of a polyimide substrate and metal pathways encapsulated in silicone rubber. Depending on the batch, either metal or CNT-silicone rubber were exposed. Female mice were implanted on the brain and under the skin (flank area) for 60 or 180 days.

After necropsy, the head was fixed in 4% neutral buffered formaldehyde. After 24h, it was stored in 70% ethanol for another 24h and then decalcified. It was then trimmed, dehydrated in ascending alcohol series and xylene. After embedding in Technovit 8100, sections with nominal thickness of 2 $\mu$ m were cut. The same procedure was performed on the implants and skin from the flank, except they were embedded in paraffin and cut into 3 $\mu$ m slices. Light microscopic examination of Hematoxylin and eosin stained slices was performed.

**Results:** During histopathological examination, both findings associated with surgery and implant-related microscopic changes were observed in sections from the head and flank. The tissue around the implant showed similar reactions in all groups. Macrophages and inflammatory cells as well as slight fibrosis were found around the implant in all animals

**Discussion:** Preparation of tissue sections including thin film implants was shown to be possible, yet the differences in material properties led to problems during cutting. However, usage of different embedding materials for the tissues worked. These first results suggest that the used CNT-Silicone rubber materials could be used in short term applications.

**Acknowledgements:** This Project is supported by Cluster of Excellence 'Hearing4All' EXC 1077 and BMBF FKZ 13GW0050B

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#### ANALYSIS OF THE CONTACT MECHANICS AND THE OPTIMIZATION POTENTIAL OF TAILORED FORMING-IMPLANTS

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**Objectives:** Tailored Forming is a new manufacturing technology to manufacture solid components out of two or more different metals. The components are joined to a hybrid semi-finished workpiece. Afterwards, a forming process is performed to improve the materials properties. This allows creating hybrid metallic parts that are adjusted to their specific loads and their field of application in comparison to parts made out of monomaterials. The potential use case of Tailored Forming-parts is still being researched. Biomedical implants are a potential field of application. In the presented study, the contact mechanics of two potential concepts for Tailored Forming hip implants were analysed.

**Methods:** Both concepts consist of a magnesium component that should be resorbed in the human body and leads to a better bone growth. A second component in the implants is used to absorb loads. In a numerical analysis the two implants were compared to a conventional implant. For the potential evaluation two load cases "walking" and "walking upstairs" were considered.

**Results:** While one concept leads to higher stresses in the implant, the other concept shows almost similar stress distributions as the conventional implant and has the additional advantage of the better bone growth due to the magnesium component.

**Discussion:** Based on this work further research on different implant concepts has to be made to give a clear statement about the potential of Tailored Forming-Implants.

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#### EFFECT OF METAL IONS ON MULTILAYER PROPERTIES AND CELL RESPONSE

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**Objectives:** The metal ions can affect differentiation of stem cells, which makes them interesting for applications in tissue engineering and regenerative in medicine. The layer-by-layer (LbL) technique is based on the



alternating adsorption of oppositely charged polyelectrolytes to form polyelectrolyte multilayers (PEM). Multilayer systems combined with metal ions of different concentration might be promising for making coatings on implants and scaffolds to regenerate tissues like bone, cartilage, and others. The purpose of this study is to investigate the influence of the concentration of metal ions on the physical properties of Polyelectrolyte multilayers (PEM), and evaluate the effect of metal ions on stem cell adhesion.

**Methods:** Polyelectrolyte multilayers (PEM) prepared by layer-by-layer technique made of polysaccharides hyaluronan (HA) as polyanion and chitosan (Chi) as polycation were additionally cross-linked by metal ions (Cu<sup>2+</sup>, Co<sup>2+</sup>, Ca<sup>2+</sup> and Fe<sup>3+</sup>), to modulate physical properties and bioactivity of multilayers to control adhesion and function of mesenchymal murine C3H10T1/2 embryonic fibroblasts. Characterization of multilayer formation and surface properties was performed by different analytical methods.

**Results:** Changes of wetting, thickness and mechanical properties of multilayers depending on concentration and type of metal ion. Most interesting, however, was the finding that metal ions like Fe<sup>3+</sup> promoted adhesion and spreading of C3H10T1/2 cell greatly on the less adhesive HA/Chi multilayer system

**Discussion:** Multilayer systems combined with metal ions can be used to regenerate tissues like bone, cartilage and others

**Acknowledgements:** This work was part of the High Performance Center Chemical and Biosystems Technology Halle/Leipzig and supported by the European Regional Development Fund (ERDF) and a grant from Martin Luther University Halle-Wittenberg for female PhD students in the final state of their project.

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### BIOSENSORS FOR IN SITU-MONITORING OF HYPOXIA IN MESENCHYMAL STEM CELLS

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**Objectives:** In vitro simulation of an in vivo environment for human stem cell research is crucial for any kind of biomedical purpose. While important factors like 3D-cultivation and physiological oxygen concentrations gain more attention in the scientific community, we still lack reliable methods to visualize the hypoxic response of cells in 2D and 3D in vitro systems. In this study we present human adipose-derived MSCs, modified with a genetically encoded hypoxia-sensor.

**Methods:** We used a lentiviral system to stably integrate the genetic construct into the chromosomal DNA of mesenchymal stem cells. To investigate the features of this novel biosensor we cultivated our cells in a 2D- and 3D-environment under various oxygen levels and evaluated the outset of biosensor-fluorescence via microscopy and flow cytometry.

**Results:** The hypoxia sensor was successfully integrated in mesenchymal stem cells and could easily be induced by cultivating the cells in a hypoxic condition. MSCs, modified with hypoxia biosensors could be cultivated up to passage 20. Trilineage differentiation of MSCs (adipogenous, osteogenous and chondrogenous differentiation) was also preserved by the cells after transfection. Using these cells we could monitor which 3D-cultivation conditions lead to hypoxic response of MSCs.

**Discussion:** Our findings can help to improve our understanding of the influence of cultivation conditions on in situ oxygen concentrations. Moreover, by choosing the right 3D cultivation system, MSC can be cultivated in vitro under physiological hypoxic conditions.

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### A NOVEL NANOCOMPOSITE HYDROGEL INK FORMULATION FOR UV-BASED 3D PRINTING

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**Objectives:** The properties of nanocomposite (NC) poly (N-isopropylacrylamide) (NIPAM) hydrogels as well as 3D printing techniques for hydrogels have gained a lot of significance in the tissue engineering community. This study aims at the development of a stable water based ink formulation for nanocomposite hydrogels usable by DLP/SLA 3D-printers in the range of 365 – 405 nm to use them e.g. for investigations in water flows.

**Methods:** Diphenyl (2,4,6-trimethylbenzoyl)phosphine oxide (as photo-initiator), Laponite XLG (as nanocomposite crosslinker), sodium dodecyl sulfate (as surfactant) and polyvinylpyrrolidone (as crystallization inhibitor) were dissolved in a N-butyl acetat and isopropanol solution at room temperature. This organic phase was then mixed with bi-distilled water and stirred for 20 minutes in a closed container to form a micro emulsion. The micro emulsion was flash frozen in liquid nitrogen and freeze dried in a Virtis Advantage Plus freeze drying unit for at least 24 h at -50 °C and 0.4 mbar. The obtained powder could be dispersed in a NIPAM/ water solution to form a stable, UV-curable ink. To show the applicability of hydrogels for investigating flows around bodies in principle, experiments in a water channel will be conducted.

**Results:** The obtained ink is suitable for the most commonly used SLA and DLP printers in the range of 365 – 405 nm. Whereas the expected curing time is much higher than the curing time of commercially available standard polymeric printing inks due to the inhibiting effect of the Laponite XLG. The applicability experiments are in preparation.

**Discussion:** NC-hydrogels obtain through this photo-polymerization method show similar mechanical properties to the standard NC-hydrogels obtained through radical polymerization with potassium peroxodisulfate.

**Acknowledgements:** This research project was partially funded by “MOBILISE – Mobility in Engineering and Science”

P2310

### ON THE CHARACTERIZATION OF THERMOMECHANICAL PROPERTIES OF NITINOL SHAPE MEMORY WIRES INTENDED FOR COCHLEAR IMPLANT ELECTRODE ARRAYS

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**Objectives:** A cochlear implant (CI) is an auditory neuroprosthesis used to restore hearing in deaf and profoundly deaf patients. It features an electrode array (EA) which needs to be inserted into the inner ear (cochlea) to enable electrical stimulation of the auditory nerve. Spatial proximity of the EA and the nerve fibers is considered beneficial. This requires a shape change of the initially straight EA to enclose the inner wall of the spiral-shaped inner ear after implantation. Thin wires made of Nitinol—a shape memory alloy (SMA)—are investigated regarding their applicability as embedded actuators to implement a shape change.

**Methods:** Nitinol wires (Ø100 µm) have been trained to a spiral shape which was derived from cross-sectional images of a human specimen as an exemplary adaption to a specific cochlea geometry. Ten different

variations of thermomechanical processing were applied by the supplier (G.RAU GmbH & Co. KG) to tailor the shape memory effect to the application specific requirements. In order to determine the resulting transformation temperatures (AS, AF) a bend and free recovery (BFR) test suitable for curved wires was developed and utilized.

**Results:** It is possible to design and fabricate spiral-shaped SMA wires which fit to a specific cochlea shape. The developed BFR test setup allows for measuring the thermomechanical properties of these actuators in an application oriented manner. This provides slightly higher but more realistic measures for AS and AF compared to differential scanning calorimetry.

**Discussion:** A complete design, manufacturing and validation process for thin spiral shape memory wires could be established. The next step is to integrate these wires into an EA and perform BFR measurements again as it is known that external forces due to stiffness of the EA alter the transformation temperatures.

**Acknowledgements:** The authors acknowledge funding by the DFG (project RA 2751/1-1).

### Artificial Lung and Tubular Implants

P241

#### SUPPRESSION OF BLOOD COAGULATION BY NITRIC OXIDE GAS ADDED TO THE SWEEP GAS DURING VENO-VEINUS EXTRACORPOREAL MEMBRANE OXYGENATION

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**Objectives:** Clot formation in the membrane oxygenator is a serious complication in long-term extracorporeal membrane oxygenation (ECMO). In the blood vessels, nitric oxide (NO) generated by nitric oxide synthase on the endothelial cells suppresses both aggregation/activation of platelets and adhesion/activation of leukocytes. Therefore, biocompatibility of the membrane oxygenator may be improved by the addition of NO gas to the sweep gas. The objective of the present study was to determine, using a rat ECMO model, whether addition of NO gas to the blood can suppress blood coagulation.

**Methods:** Male Sprague Dawley rats weighing 350 to 400 g were used for the experiments. After the rats were anesthetized, extracorporeal circulation was established by guiding the blood from the right jugular vein into the left femoral vein. Heparin was administered by bolus injection (0.7 unit/g-rat) only at the start of the ECMO. Polymethyl pentene membranes were used for the oxygenator. The sweep gas (NO: 0, 100 or 200 ppm, O<sub>2</sub>: 30%, CO<sub>2</sub>: 5%, N<sub>2</sub>: balance) was added to the blood via the oxygenator. During the experiment, the arterial pressure, oxygenator inlet pressure, venous pressure, activated whole blood clotting time (ACT), and methemoglobin (Met-Hb) concentration were measured. After circulation, the hemoglobin (Hb) content and lactate dehydrogenase (LDH) activity in the eluate from the residual blood in the oxygenator were measured.

**Results:** Extracorporeal circulation was stably performed for 4 hours. There were no differences in the ACT during the experiment among the three groups. The amount of Met-Hb was below 10% in the NO group. The Hb content and LDH activity in the eluate were significantly lower in the 200-ppm NO group than those in the 0 ppm NO.

**Discussion:** The amount of Met-Hb in the NO group indicates that there is no adverse effect related to Met-Hb. The Hb content and LDH activity indicate that the NO contained in the sweep gas inhibited blood coagulation in this rat extracorporeal circulation model.

P242

#### FULLY RESOLVED CFD SIMULATION OF THE CO<sub>2</sub> TRANSPORT IN A HOLLOW FIBER MEMBRANE OXYGENATOR PACKING

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**Objectives:** Membrane oxygenators are an indispensable part of critical care medicine. Though necessary to supply sufficient gas exchange, the high intrinsic surface introduced by the hollow fiber packing has serious side effects on blood platelet parameters. To reduce these side effects the membrane surface must be minimized and gas exchange improved.

**Methods:** Computational fluid dynamics (CFD) can support oxygenator optimization and supplement experimental data by delivering a spatial and temporal resolution of the gas exchange. While current research mostly focusses on the gas transport in the blood flow, this work presents a fully resolved CFD approach including transmembrane transport as well as convective and diffusive blood gas transport on shell- and lumen-side of the hollow fibers.

**Results:** CO<sub>2</sub> transport in a packing segment of a prototype hollow fiber module was fully resolved and simulated utilizing an inhouse solver membraneFoam based on the open source CFD code OpenFOAM®. Simulation results show a CO<sub>2</sub> partial pressure decline from 50 to 15 mmHg in the laminar boundary layer and an additional drop of 12 mmHg at the selective membrane surface. Boundary conditions for the gas transport simulations were computed by blood flow simulations of the whole module. Simulation results were compared to in vitro tests comprising measurements of CO<sub>2</sub> exchange performance and blood side pressure drop of the prototype module.

**Discussion:** Flow simulations predict the experimentally determined pressure drop of 68 mmHg at blood flow rates of 1280 mL/min accurately. The specific CO<sub>2</sub> exchange rate of 220 mL STP/min/m<sup>2</sup> is over-predicted due to the reduction of the whole packing to an idealized packing segment. Nevertheless, CFD allows for a structured optimization of membrane oxygenators as design changes can be efficiently investigated.

**Acknowledgements:** This work is supported by the Austrian Research Promotion Agency (FFG). Project: MILL – Minimal Invasive Liquid Lung. Project number: 857859.

P243

#### METHODS FOR LUNG DOWNSIZING AS SURGICAL INTERVENTIONS DURING EX VIVO LUNG PERFUSION IN A NEW CUSTOM-MADE MODEL

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**Objectives:** Ex vivo lung perfusion (EVLP) is a technique to evaluate lungs before transplantation. As a tool for experimental research, this method allows organ modification and assessment. However, the costs are high. The aim of this study was to establish a low-cost EVLP model on the one hand and to investigate the feasibility of surgical graft downsizing during

EVLP on the other hand. We compared a lobectomy, a stapled wedge and a sutured wedge resection during EVLP.

**Methods:** Pigs of 60 kg weight were used for organ harvesting. After cardiac death lungs were retrieved, flushed with Perfadex and perfused with autologous blood for 4 hours of EVLP. The circuit was assembled from an ECMO system consisting of a Deltastream DP2 pump (Medos), an Affinity Fusion oxygenator (Medtronic) and a hardshell reservoir. After 90 minutes of perfusion a lobectomy was performed. At intervals of 30 minutes, a stapled wedge resection and a sutured wedge resection were performed. Air leak and the loss of blood were measured. The function of the lung was monitored including pulmonary artery pressure and blood gas analysis.

**Results:** 20 porcine lungs were used to establish a stable model. Another 7 lungs underwent surgical interventions. The blood loss after lobectomy ( $3,14 \pm 4,14$  ml/min) and after stapled wedge resection ( $5,29 \pm 8,1$  ml/min) was lower than after sutured wedge resection ( $33 \pm 17,36$  ml/min). There was no major difference in air leak after the surgical interventions (lobectomy:  $0,06 \pm 0,16$  l/min vs. stapler:  $0,03 \pm 0,09$  l/min vs. suture:  $0,04 \pm 0,1$  l/min). The oxygenation performance was satisfactory. The pulmonary artery pressure was in a physiological range. Bleeding into the parenchyma after suture was higher compared to the other interventions.

**Discussion:** The low-cost custom-made EVLP model presented here is feasible and stable. Surgical interventions during EVLP are possible. Performing a lobectomy and a wedge resection is superior to a wedge resection with suture regarding to the blood loss.

P244

#### CONSIDERATION OF THE GAS FLOW DURING THE DEVELOPMENT OF A NOVEL BLOOD GAS EXCHANGER FOR CHRONIC USE

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**Objectives:** The aim of the study is to analyse and modify the gas transfer properties of a novel PMP fiber blood gas exchanger with a cylindrical shaped blood flow channel by improving the sweep gas flow profile through the fiber bundle while considering the fiber length in blood contact.

**Methods:** A test set-up has been developed to visualize the gas flow through the fiber bundle by means of steam. In an in-vitro setting with porcine blood the gas transfer rate has been measured subsequently. The cylindrical blood flow geometry and the fact that the fibers are stacked with an offset of 90° onto each other results in fibers with various effective lengths. For the study, various prototypes of gas inlets with different inlet geometries have been designed, manufactured and evaluated regarding their gas transfer rates.

**Results:** The different designs resulted in the aimed flow profiles during the flow visualization. Selected prototypes have been tested with blood and the gas transfer rates have been determined. However, the modified designs with improved gas flow properties do not significantly impact the gas transfer rates measured with blood.

**Discussion:** Two possible explanations can be derived from the results which may be tested in the course of further studies. Firstly, it is possible that the current gas inlet is already sufficiently adapted to the cylindrical blood flow geometry and fibers of different effective length. Secondly, it could be considered that side parameters such as the diffusive transfer of water vapour from the blood side to the gas side have a superseding influence on the gas flow profile. In conclusion, the gas transfer rates

cannot be significantly increased by manipulating the gas flow profile through the fiber bundle. Nevertheless, the blood gas exchanger achieves already comparable transfer rates to products on the market but has reduced PMP membrane surface.

P245

#### TOTAL LIQUID VENTILATION WITH OXYGEN MICRO/NANO BUBBLE DISPERSED SALINE PREVENTS FROM LIPOPOLYSACCHARIDE-INDUCED ACUTE LUNG INJURY IN RATS

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**Objectives:** To examine the efficacy of the short-time total liquid ventilation (TLV) with oxygen micro/nano bubble dispersed saline for severe and lethal respiratory failure (experiment 1 and 2, respectively) induced by intratracheal administration of lipopolysaccharide (LPS).

**Methods:** In experiment 1, fifteen rats were divided into three groups: LPS+TLV group (treatment group) received 5-min TLV 20 min after LPS (5 mg/kg) administration, LPS+MGV group received 5-min mechanical gas ventilation (MGV) after the LPS administration, and PBS+MGV group received 5-min MGV 20 min after administration of phosphate buffered saline (PBS) instead of LPS. Ventilation weaning was performed after respiratory management for three hours. At two days after each treatment, hemodynamics and blood gas parameters were measured and the rats were subsequently sacrificed. The excised lungs were used for histological analysis and bronchoalveolar lavage (BAL). In experiment 2, ten rats were divided into two groups: LPS+TLV group and LPS+MGV group. Ten mg/kg LPS was used for inducing lethal acute lung injury and the survival rate was used for the primary outcome.

**Results:** In experiment 1, significant differences were confirmed between LPS+TLV group and LPS+MGV group in the value of blood oxygen, weight loss ratio, and inflammatory cytokines levels in BAL-fluid ( $p < 0.05$ ). On the other hand, there was a significant difference only in arterial oxygen pressure between LPS+TLV group and PBS+MGV group. Severe inflammation in LPS+MGV group was also confirmed in the histological analysis. In experiment 2, all rats in LPS+MGV group died within two days, whereas 80% of the rats in LPS+TLV group survived for one week. Moreover, the conditions of the survival rats showed normal in the pulmonary function and the alveolar structure after one week.

**Discussion:** It is indicated that 5-min TLV with oxygen micro/nano bubble dispersed saline after LPS administration prevented severe respiratory failure and improved the survival rate dramatically without any disorders.

P246

#### IMPROVED DRAINAGE CANNULA DESIGN TO AVOID THROMBOSIS IN VENO-ARTERIAL ECMO

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**Objectives:** The aim of this paper is to improve the design of the current commercial cannula being used by clinicians in order to minimise the blood flow stasis and thus avoid thrombosis in ECMO drainage cannula

**Methods:** A CFD simulation is used in order to determine the optimum design for the drainage cannula. The improved cannula design was fabricated and validated in-vitro using particle image velocimetry.

**Results:** 16 different cannula designs were simulated and results were analysed in terms of velocity and mass flow rate at side holes and different sections of the cannula. Different parameters including side holes diameter, number, spacing, distance to the cannula tip, and angle relative to flow direction were investigated. Simulation results were in agreement with PIV.

**Discussion:** This study demonstrated that the improved drainage cannula can significantly reduce blood flow stasis, and potentially thrombosis, in patients receiving ECMO.

P247

### PERISTALTIC FUNCTION OF THE ARTIFICIAL ESOPHAGUS BY THE USE OF THE ORIGAMI STRUCTURE WITH THE SHAPE MEMORY ALLOY ACTUATOR.

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**Objectives:** Long term survival rate after the resection for the esophageal carcinoma has not been satisfactory. One of the most important topics in the area of the esophageal cancer is the reconstruction of the esophageal tract. If the artificial esophagus can be constructed, this technology will be beneficial for patients with esophageal cancer in the advanced stage.

**Methods:** In the previous reports, artificial esophagus with shape memory (SMA) alloy actuator had been reported. However, total occlusion of the esophageal tract had been difficult. In this study, Origami engineering technology had been used for the peristaltic motion. Triangle shape of the Origami structure had been designed in this study for the total occlusion of the esophageal tracts to prevent the gastroesophageal reflux. The corner of the triangle had been pulled by SMA actuators following the diagonal lines for the folding power.

**Results:** In the design concept of this artificial esophagus, the artificial esophageal tracts will be sutured to the human's esophagus. In this stage, the artificial esophageal tracts were consisted by the artificial vessel's material, and in the next stage regenerative esophagus will be the candidates. Esophageal tracts will be covered with triangle shape tracts with SMA actuator to embody the peristalsis. In the results in this study, total occlusion of the artificial esophageal tracts had been embodied by the use of the Origami structure. Through the arrangement of the triangle shape origami structures, co-driving will be embodied for the peristalsis function.

**Discussion:** Until now, there had been no success in achieving peristalsis motion of the artificial esophagus. For the application of the human esophageal tracts, more study is necessary. This study was the first step for the application of the new artificial esophagus in the world for the achievement of the totally occluded peristalsis.

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### APPLICATION OF IN VIVO TISSUE ENGINEERED DECELLULARIZED CONNECTIVE TISSUE FOR CARDIOVASCULAR GRAFTS

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**Objectives:** We have developed in vivo tissue engineered autologous vascular grafts constructed in the subcutaneous of the recipient body. However, since the formation of the vascular grafts depends on the conditions of recipients including high risk or immature patients, immaturity in the fabricated tissues might be problematic for the severely diseased patients because of their suppressed regenerative activity. Therefore, possibility of the xenogeneic or the allogeneic implantation of the grafts should be evaluated. The objective of this study is to fabricate cardiovascular grafts using xenogeneic or allogeneic animals.

**Methods:** Silicone rod molds were placed into subcutaneous pouches of beagle dogs, and after 4 weeks the implants with their surrounded connective tissues were harvested. Those were decellularized with detergents and stored at -20 °C for 1 week. Decellularized tubular connective tissues (internal diameter: 2 mm) were xeno-transplanted to abdominal aorta of the rats. Decellularized tubular connective tissues (internal diameter: 5 mm) were cut open and trimmed to elliptical sheets of 15 x 8 mm, they were allo-transplanted to carotid arteries of other beagle dogs as vascular patches.

**Results:** Both xenogeneic vascular grafts and allogeneic patch grafts performed well after transplantation, and the luminal surfaces after resection were very smooth. Histological evaluation also showed host cells infiltration into the grafts.

**Discussion:** Decellularized xenogeneic and allogeneic connective tissue membranes could be ideal vascular grafts.

P249

### INHALED DRUG AIRFLOW PATTERNS AND PARTICLES DEPOSITION IN THE PEDIATRIC RESPIRATORY TRACT – NUMERICAL FLOW INVESTIGATIONS

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**Objectives:** The effectiveness of the inhaled drugs is strictly related to the areas which are reachable by the drug particles. Unless the particles reach the desired part of the bronchial tree, their influence might not meet the required expectations and consequently the disease progress might not be stopped. Therefore, the primary objective of this research was to analyze the airflow patterns and particles deposition of a standard inhaled drug, commonly used during the treatment of asthma, with the use of computational fluid dynamics (CFD). The study was devoted to the analysis of the particles diameter influence on their deposition areas within the entire respiratory tract.

**Methods:** Two patient-specific respiratory tract models, for 6 and 12-year-old patients, were reconstructed basing on the DICOM image sets obtained during the Computed Tomography Examinations. The reconstruction of the digital models was performed within the Mimics Research 20.0 program, whereas the meshes were prepared with the use of the Ansys ICEM package. Numerical analyses were carried out as stationary ones with the constant inflow of the particles of various diameters (within the range of 1.0-50.0 μm).

**Results:** It was proven that depending on the particles size, their deposition within the respiratory tract varies significantly. The vast majority of the particles with diameters over 20 μm are gathered on the walls of the throat, whereas particles of diameters 5-15 μm are accumulating mainly on the trachea walls, leaving the alveoli insufficiently supplied with the drug particles. Similar phenomena could be observed for both investigated cases of the reconstructed geometries.

**Discussion:** The significant changes of the drug particle distribution resulting from the various diameters of the particles indicate that the inhaled drug size cannot be treated as negligible factor during the drug spraying. Improper distribution of the particles, i.e. their aggregation on the wall of the throat and trachea, might not inhibit the symptoms of the asthma.

P2410

### CONTRIBUTION OF HUMAN STEM CELL-DERIVED CARDIOMYOCYTE SUBTYPES TO BIOARTIFICIAL CARDIAC TISSUE FORMATION AND FUNCTION

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**Objectives:** The transplantation of human induced pluripotent stem cell (hiPSC)-derived grafts represents an alternative treatment option for post-myocardial infarction patients. So far, myocardial tissue engineering mainly focused on the generation of ventricular-like tissues providing sufficient contractile function. Atrial- and pacemaker-like cardiomyocytes (CMs) are hypothesized to have a negative effect on tissue function, as they could lead to a lower force development and to arrhythmias after transplantation, respectively. Therefore, we aim to characterize subtype composition in the differentiated CM cultures, as well as in generated tissues thereof and analyze tissue functionality.

**Methods:** The hiPSC-CMs are then mixed with human fibroblasts and a matrix to generate bioartificial cardiac tissues (BCTs) using our established bioreactor technology. In order to assess the ratio of individual iPSC-derived CM subtypes, flow cytometry and immunofluorescence (IF) stainings with antibodies targeting subtype-specific markers (MLC2v, COUPTF-2, and MLC2a) were performed.

**Results:** First results revealed a high percentage (~80-90%) of MLC2v positive cells in differentiated CM populations. The remaining cells stained positive for atrial markers (COUPTF-2, MLC2a). Functional measurements mainly focused on BCT contractility and showed forces similar to the native myocardium (~6.5 mN/mm<sup>2</sup>). In addition, we observed that a higher proportion of ventricular-like CMs leads to higher contractile BCT function.

**Discussion:** Taken together, these molecular and functional results reveal that our differentiation protocol led to the formation of mainly ventricular-like CMs and that BCTs generated thereof show typical characteristics of myocardial tissues. For future ventricular-like tissue replacement therapy, we aim to perform a risk assessment for "contaminating" cardiac cell types with respect to both BCT function and arrhythmias.

## Symposia

### Nikkiso Lunch Symposium on Artificial Pancreas

LS211

### CHAIRMAN MESSAGE: TECHNICAL SOLUTIONS FOR A BEDSIDE TYPE ARTIFICIAL PANCREAS AND FUTURE PERSPECTIVES

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**Objectives:** Perioperative tight glycemic control (TGC) and management is of paramount importance during perioperative treatment of patients with multiorgan failure. Recent results have shown that such interoperative TGC led to an improvement of morbidity and mortality and to a prevention of Surgical site infection (SSI) in ICU patients. Consequently, a

user-friendly device combining glucose analysis and delivery of glucose and insulin should allow for better therapies.

**Methods:** Based on an already existing artificial pancreas device (STG-22), a new artificial endocrine pancreas system (STG-55) was developed. With a battery incorporated, it uses again a closed loop analytical system. Due to its in-built sensors, the closed-loop system allows for a quick and easy feed-back analysis of patient data through already established algorithms for glucose and insulin infusion. With the help of a disposable modular tubing circuit blood sampling from the patient's vein is realized and assessed by an automatically calibrated sensor. The device exhibits a maneuverable weight of only 36 kg and can be set-up within a single hour.

**Results:** Clinical trials with STG-55, in Japan (Kochi University Hospital) have proven its re-liable practicability. Glucose monitoring was stable and results comparable to the analyses of an independent glucose analyzer with a high correlation coefficient. Due to appropriate insulin administration, no hypoglycemia was observed in patients under investigation.

**Discussion:** The advent of new technological tools, such as miniaturized analytical devices in combination with the application of patient-specific precise algorithms allows for a strict and successful therapy without hypoglycemia in perioperative patients. With the new design and concept of STG-55, the set-up time for the artificial pancreas can be reduced down to 30% of preexisting devices, which finally makes a glucose and insulin management in both the interoperative and the ICU user friendly.

### Smart Pumping with Ventricular Assist Devices

S112

### HEMODYNAMIC STATUS AND DEMAND OF LVAD-PATIENTS DURING EXERCISE

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**Objectives:** Over time left ventricular assist devices (LVAD) have become an alternative to heart transplantation because of enormous technical development and miniaturization. Implantation of these systems has been proven to lead to considerably improved survival, with an increase in quality of life. Following implantation, most patients display a considerable improvement in physical capacity, measured according to the New York Heart Association (NYHA) classification, even though exercise tolerance levels following the implantation of an LVAD are still considerably restricted.

**Methods:** Current state of scientific knowledge with respect to the physical capacity of patients with terminal heart failure after LVAD implantation is described at rest and during exercise. The significance of new diagnostic tools, such as the non-invasive inert gas rebreathing method for measurement of cardiac output and arteriovenous oxygen difference (AVDO<sub>2</sub>) in assessment of the performance of LVAD patients is discussed.

**Results:** Pump flow across the LVAD can be increased to a certain degree during exercise and normal everyday activities via two mechanisms: exercise-dependent tachycardia and increase of enddiastolic pressure in the left ventricle in conjunction with increased preloading. The share of the native left ventricle in systemic perfusion can also increase during exercise. Here, left-ventricular pressure exceeds systemic blood pressure and the aortic valve opens. The contractile reserve of the myocardium does, of course, play a crucial role.

**Discussion:** Ultimately, the cardiopulmonary capacity of LVAD patients is restricted to a higher degree during exercise, even after implantation. Larger studies are required to clarify the extent to which longer-term physical exercise training after discharge from rehabilitation and/or an increase in the number of pump rotations can lead to increased physical capacity in LVAD patients.

S113

### MODELLING THE HEMODYNAMICS OF LVAD PATIENTS AT REST AND EXERCISE

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**Objectives:** Exercise capacity in patients with a continuous-flow left ventricular assist device (LVAD) is 50% compared to healthy subjects. Some patients show a better performance than others. Aim of this work is to investigate the factors responsible of this inter-subject variability.

**Methods:** A cardiorespiratory simulator (CRS) was developed and combined with a model of the HVAD running at 2700 rpm to reproduce an "average" LVAD patients hemodynamic at rest and at exercise. The CRS was validated using the average hemodynamic data of >200 LVAD patients from the literature. Starting from this "average" LVAD profile, a sensitivity analysis was conducted by simulating a left/right ventricular contractility (Elmax/Ermax) and heart rate (HR) 20% higher than value usually observed at exercise.

**Results:** The simulated "average" LVAD profile at rest is in full support with a total cardiac output (CO) of 4.8 l/min and a wedge pressure (Pw) of 13 mmHg. At exercise Pw increases to 26 mmHg and CO increases to 6.8 l/min, of which 5.3 l/min pumped by the LVAD (QLVAD) and 1.5 l/min by the ventricle (QLV). An increased Elmax at exercise assures lower Pw (22 mmHg) and higher CO (7.4 l/min) due to a better contribution of the ventricle (QVAD=4.7 l/min, QLV=2.7 l/min). An improved HR also helps to sustain a better CO (7.1 l/min) as a combined effect of both pumps (QVAD= 5.4 l/min, QLV=1.7 l/min). A better Ermax induces a smaller improvement in CO (7.0 l/min) due to the increased LVAD support (QVAD=5.5 l/min, QLV=1.5 l/min), however Pw reaches 31 mmHg.

**Discussion:** A better left ventricular inotropic response is mostly effective in accommodating a higher CO at exercise. A better right ventricular function has mild effects on CO and can increase Pw if not sustained by a good ejection on the left side. These results should be taken into account for the design of a physiological LVAD speed controller, tailored on specific patient's needs.

**Acknowledgements:** Frans Van de Werf scholarship.

S115

### PHYSIOLOGIC CONTROL OF CARDIAC ASSIST DEVICES: ACHIEVEMENTS AND CHALLENGES

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**Objectives:** Left Ventricular Assist Device (LVAD) therapy has become an accepted treatment for end-stage heart-failure. While patient management has improved over the past decades, limitations in caregiver resources necessitate efficient allocation. Decisions on the optimal setting of LVAD support currently occurs in intervals ranging from a few weeks to a few months. However, the optimal setting for LVAD support may change multiple times per minute. Thus, automation is the only viable solution to this problem. Physiological control promises to integrate the cardiac assist device into patient physiology even more closely,

reacting to varying demands or specifying the load for training of the heart muscle. One important design consideration is the choice of feedback variable.

**Methods:** Literature of the past two decades was collected and the presented physiological control concepts were clustered into categories corresponding to functional similarity of feedback variables.

**Results:** It was found that most literature could be summarized into five categories. Control algorithms either used preload, heart rate, ventricular contraction, or afterload as feedback variables or modified the flow-rate/headpressure relation.

**Discussion:** Each of these categories contains information about the cardiac state. While preload variables, most closely resemble the native functioning of the left ventricle, they are inherently difficult to measure without using additional sensors. Chronotropic incompetence might be a limiting factor for heart rate based control. Ventricular functioning might fluctuate over time due to changes in inotropy while afterload is heavily regulated by other autoregulatory mechanisms. The headpressure/flowrate relation suffers from uncertainty regarding the origin of pressure changes.

Combining elements of these five categories into one control concept might prove beneficial, as different information could be extracted for the controller, which could then be tuned by the physician.

### Blood Trauma in Artificial Organs

S121

#### CHANGES IN THE PHYSICAL PROPERTIES OF BLOOD AND THE MICROCIRCULATION DURING CARDIOPULMONARY BYPASS

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**Objectives:** Blood trauma is an unintended consequence of mechanical circulatory support. Evidence indicates that exposure to high shears may induce sublethal changes to blood cells and also overt cell destruction. To determine whether such changes in blood properties have a functional impact on microcirculatory function, we examined the effect of surgery requiring cardiopulmonary bypass on the rheological properties of blood and also direct assessments of cell flux in the microcirculation.

**Methods:** Thirty individuals requiring elective coronary artery bypass graft (n = 15) or valve surgery (n = 15) volunteered to participate. Dependent variables were collected prior to use of cardiopulmonary bypass, after 60 min of being on pump, and in the post-surgical period. Blood analyses included measurements of blood and plasma viscosity, red cell deformability, red cell aggregability, mechanical sensitivity of red cells, and levels of haemolysis, among others. Microcirculatory function was assessed using incident dark field visualisation of the sublingual vessels.

**Results and Discussion:** Initial findings of this project indicate that blood viscosity and red cell aggregability are significantly affected by 60 min of cardiopulmonary bypass; these measures do not restore following the early post-surgical period. Microcirculatory assessments reveal altered transit and velocities of red cells, potentially indicating a link between biophysical assessments of blood and microcirculatory function. Extended presentation of this ongoing study will be presented, and discussed in the context of the secondary complications that still plague circulatory support patients.

S122

### A PORE MODEL FOR HEMOGLOBIN TRANSPORT COMBINED WITH THE STRAIN-BASED HEMOLYSIS ESTIMATION

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**Objectives:** The Eulerian version of the strain-based hemolysis model has already shown to predict different critical regions in blood pumps compared to the commonly used stress-based models. Where the stress-based model assumes an instantaneous deformation of red blood cells (RBCs) due to the action of fluid forces, the strain-based model is able to estimate the viscoelastic deformation of RBCs. Thus, the effect of exposure time is considered in a natural way. However, both models are based on the empirical power law assumption for hemoglobin transport and turned out to overpredict hemolysis compared to experimental data. In order to improve the prediction of free-plasma hemoglobin, the strain-based model is combined with a multiscale model for hemoglobin transport. The model is able to account for pore formation in the RBC membrane and the resulting hemoglobin mass transfer into the blood plasma. Thus, the model does not rely on the power law assumption.

**Methods:** The formation of pores is computed based on an energy balance of the stretched RBC membrane, where an area strain can be computed by the tensor equation of the strain-based model. The hemoglobin release is modeled by a transport equation with a mass transfer coefficient as a function of shear, which can be fitted to the data of blood shearing experiments. As an application, the model is used for a benchmark blood pump provided by the FDA.

**Results:** The estimated total hemolytic performance for the benchmark blood pump is significantly reduced compared to the power law version of the strain-based model. The new results are in good agreement with the measured data by the FDA.

**Discussion:** The multiscale model for hemoglobin transport turns out to be a useful extension of the strain-based hemolysis model, allowing for an overall more physical model description of the hemolysis process. Application to different devices and blood shearing experiments should be investigated in the future, in order to study the parameter sensitivity.

S123

### ABRUPT CHANGE OF SHEAR STRESS AS ADDITIONAL HEMOLYSIS FACTOR

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Conventional hemolysis researches were performed under mainly constant shear stress, however, real flow in vivo would be under fluctuating shear stress. In addition, several conventional numerical simulations predicted abrupt shear stress fluctuation in the blood pumps in short time period. Furthermore, it was also reported that erythrocyte showed its unique viscoelastic shape change response to sinusoidal changing shear stress. Based on such information, we hypothesized that abrupt shear change would be additional hemolysis factor in addition to conventional shear stress and its exposure time. Therefore, the purpose of this study was to examine the feasibility of such our hypothesis. We developed special cylindrical shear chamber which can generate varying shear stress by controlling the rotational speed. This study used the shear device to generate several trapezoidal waveforms of shear stresses. In such shear fluctuation, the maximal shear stress of approximate 22.5 Pa were loaded to

porcine whole blood for 5.0 seconds. And the time series integrated shear stress was set to equal between abrupt and slow shear change conditions. Then, generated hemolysis levels were compared between them. Each comparative studies were performed for twelve times. Furthermore, such studies were repeated under several different shear change speeds. The result of our all comparison showed greater hemolysis amount at abruptly fluctuating shear condition. Such results successfully validated our hypothesis.

S124

### INVESTIGATION OF THE BLOOD DAMAGE IN DIFFERENT GAP GEOMETRIES USING MICROCHANNELS

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**Objectives:** The long-term usage of rotary blood pumps is limited by the high amount of blood damage, which leads to a high number of adverse events. Most of the blood damage is generated in the gap between the rotor blades and the housing wall, the area with the highest stress load and very short exposure times. The damage is caused by both shear and normal forces on the blood components. The objective is to investigate the blood damage in this region by examining different gap geometries. The focus is the damage caused by high dynamic normal stresses and the comparison with the blood damage models.

**Methods:** A section of the blade gap of an axial rotary blood pump was reconstructed on a microchannel chip, which is sealed with siliconized cover glasses. In additional channels the inlet and outlet rounding are varied between 0.05 and 0.6 mm. The gap length and width are kept the same for all channels. A channel without a blade gap is integrated to determine the damage caused by the test stand. Flow simulations are carried out to determine the occurring stresses. The test stand consists of a syringe pump that transports 20 mL of human blood back and forth through the microchannels for up to 6 hours. Hemolysis is measured every half hour.

**Results:** With the test stand it is possible to investigate the influence of different gap geometries on the blood damage under controlled conditions. The experiments demonstrated the influence of different inlet and outlet geometries on the hemolysis. The measured hemolysis was compared with the damage models.

**Discussion:** With the test stand it is possible to simulate the stress loads in blade gaps on human blood. The influence of the high dynamic normal stresses on hemolysis can also be determined through variations of the inlet and outlet. It is therefore a well-suited tool for improving the corresponding damage models in the area of high dynamic stress loads. The findings allow the optimization of the region to further reduce blood damage caused by rotary blood pumps.

S125

### A NOVEL METHOD FOR HEMOLYSIS PREDICTION IN CFD

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We discuss a novel method for hemolysis prediction in computational fluid dynamics, which avoids potential problems inherent to the traditionally used Eulerian and Lagrangian methods.

The traditionally used Eulerian method computes a hemolysis index (HI) at the flow outlet by solving a transport partial differential equation (PDE) where the source term equals shear stress raised to some power

(which is determined empirically), and then looks at the HI transported to the flow outlet. The largest contribution to HI typically occurs at the flow boundaries (walls), which is precisely the location where numerical noise is most likely to be inadvertently introduced, e.g., from sharp edges in the wall or from low quality mesh elements. This can cause errors in the Eulerian method because its PDE lacks a diffusion term, and consequently any numerical noise is transported to, and accumulates at, the flow outlet surface.

We have not seen this potential problem occurring when using a Lagrangian method, although theoretically it could still occur. Regardless, a major disadvantage of the Lagrangian method is a large computational cost required to achieve reasonable accuracy.

Instead of using one of the two traditional methods, we use a volume integral where the source term includes both shear stress and flow speed. In situations with sufficient mixing, such as in an LVAD, this approach predicts blood damage at least as well as either of the other methods, for a fraction of the computational cost, and avoids the numerical problems. The method also lends itself better to frozen rotor simulations, while the traditional methods prefer more costly time-dependent simulations. We present an example where the method is used to evaluate potential changes to a HM3-like rotor design.

S126

#### ELEVATED BLOOD TRAUMA POTENTIAL OF THE HVAD PUMP IN PEDIATRIC PATIENTS

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**Objectives:** Mechanical circulatory support (MCS) has become a standard therapy for adult end-stage heart failure patients. For pediatric patients, technological development lags behind with no currently approved implantable rotary blood pump. As an alternative, the HeartWare HVAD, originally designed for adults, is increasingly used in pediatric patients. The aim of this multicenter study was to assess *in-silico*, *in-vitro* and *in-vivo* the blood trauma potential of this pump in pediatric application.

**Methods:** Blood trauma potential of the HVAD was investigated *in-silico* and *in-vitro* at an adult and pediatric operating point (5L/min and 2.5L/min at 2800rpm and 2200rpm, respectively). The flow was simulated by computational fluid dynamics and analyzed regarding flow structures, shear stresses and washout. Hemolysis was assessed with pumps circulating bovine blood in a temperate flow circuit. Clinical outcome and indicators for *in-vivo* blood trauma were investigated retrospectively in 14 pediatric HVAD patients (age 11.3±4.8years).

**Results:** In the pediatric conditions, simulations predicted elevated mechanical stress profile below 50mPa, more stagnant flow field, with longer washout times within the pump. *In-vitro* measurements revealed an increased normalized index of hemolysis (NIH = 17.5 mg/100L vs. 8.2 mg/100L, (p=0.0021)). In the retrospective *in vivo* analysis, LDH and D-Dimer values were 1.5 and 3-fold elevated, respectively, compared to adult HVAD patients. Major bleedings were observed in 42.9%, suspected pump thrombosis and neurologic dysfunction in 14.3% of all patients.

**Discussion:** The HVAD, operated at lower speeds and flows, induces elevated blood trauma. These results highlight the need for specifically adapted ventricular assist devices, optimized for the pediatric population. Further studies are required to assess the clinical implications of these findings.

#### IFAO - Frontiers of Biomaterial with Surface Treatment and Biological Tissues

S131

#### FRONTIERS OF BIOMATERIALS: CELL SEPARATION AND BIOLOGICAL TISSUE HYBRIDS

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**Objectives:** In this study, two attempts of new biomaterial development based on the latest results of cell biology and tissue engineering are reported. One is specific cell separation using DNA as linker, and another is novel hybrid materials constructed by decellularized tissues complexed with synthetic polymer.

**Methods:** Antibody was immobilized via desthiobiotin-avidin interaction. Single strand DNA (20mer) was chemically immobilized on the surface and then antibody (anti-mouse CD45, mCD45) modified with the complementary single strand DNA was immobilized on the surface through DNA duplex formation. Decellularized porcine dermis was prepared and lyophilized. After that, methyl methacrylate (MMA) monomer were immersed in stages, then polymerization was proceeded. The obtained hybrid material was analyzed by tensile strength and SEM observation.

**Results:** Cells were also adhered on the surface at 37 degrees C and detached by 4 degrees C incubation with remaining cells that interacted with the surface via antibody-antigen interaction. The adhered cells were released by DNase treatment. These results suggest that cells can be selectively captured and collected by using the surface that immobilizes an antibody via dissociation molecules. The monomer absorption depends strongly on the tissue structure. Gradient-type decellularized dermis-poly(methyl methacrylate) complex was prepared by controlling the permeation time of the methyl methacrylate monomer. The mechanical strength of this complex gradually increased from the dermis side to the polymer side.

**Discussion:** Developing new biomaterials combining new knowledge among different science discipline is an indispensable methodology for the next generation of medical development. The materials presented here are example of these biomaterials, and show the direction of future biomaterial development.

**Acknowledgements:** Author thanks Drs. T. Kimura, Y. Hashimoto, N. Nakamura, Y. Zhang, and S. Funamoto.

S132

#### ENGINEERING OF BIOACTIVE SURFACE COATINGS AND HYDROGELS WITH POLYSACCHARIDES

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**Objectives:** Polysaccharides belong to the most abundant biomaterials on earth. They form structural elements of plant and animal tissues, but many of them have also important regulatory functions towards cells, tissues and organs. Glycosaminoglycans (GAG) and other carboxylated



and sulfated polysaccharides possess a bioactivity that is due to their high affinity to a plethora of proteins forming insoluble and soluble components of cell environments, but also direct interaction with cell receptors.

**Methods:** Making surface coatings for implants from natural or semisynthetic GAG that guide cell behavior requires different strategies of covalent or physical attachment. Simple covalent binding mechanisms can be based on oxidation of pendant hydroxyl groups of monosaccharide subunits or introduction of reactive side groups like thiols that permit direct coupling to surfaces.

**Results:** Physical can exploit the inherent charge of these molecules that permits formation of multilayers kept together by ion pairing, but also intrinsic cross-linking of activated GAG. Both mechanism can be also used to form 3D structures as biocompatible in situ gelling hydrogels that permit embedding of growth factors and cells.

**Discussion:** These 2D and 3D systems are instructive controlling cell spreading, growth and differentiation, which will be shown with examples on chondrogenic and osteogenic differentiation of mesenchymal stem and other cells.

**Acknowledgements:** This work was supported by Deutsche Forschungsgemeinschaft grant Gr 1290/11-1 & Gr1290/12-1 and Bundesland Sachsen-Anhalt in the frame of Leistungszentrum Chemie- und Biosystemtechnik (project N<sup>o</sup>. H01127247).

S133

#### NOVEL TISSUE ADHESION METHOD USING LOW INTEGRATED ENERGY

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**Objectives:** A new method to adhere biological tissue to another or to metal such as stainless steel using integrated low-level energy sources, heat, pressure, and vibration is proposed.

**Methods:** Heat, pressure, and vibration are simultaneously delivered by pressing the porcine aorta tissues on the vibration plate with the heated tip. Tensile tests on adhered specimens were performed to determine adhesion characteristics to adhesion temperature, time, pressure, and vibration. The effect of metal surface coating, fluorine-doped DLC coating, on the adhesion strength was examined. In one application, the inlet port of a ventricular assist device adhered to a porcine ventricle muscle by applying the novel method with a temperature of 80 degrees Centigrade for 120 seconds. Sufficient adhesion was observed without additional vibration. In a second application, the vessel adhesion apparatus was developed to connect a bypass graft to a coronary artery. The device has both a heating and pressurizing part on the tip, which was used to attach the bypass graft to the coronary artery by applying heat with a temperature of 120 degrees Centigrade for 120 seconds.

**Results and Discussion:** The maximal shear tensile strength using the proposed novel adhesion method was 200 kPa, which is half the strength of the porcine aorta. Adhesion strength increased in proportion to temperature, time, and pressure. The adhesion strength can be controlled by surface treatment of metals. In the first application, the inlet port adhered to the ventricle muscle with a shear tensile strength of 91 kPa, which is enough to keep the pump attached to the ventricle. In the second application, the bypass graft adhered to the coronary artery with a

shear tensile strength of 50 kPa, which is sufficient to maintain attachment when exposed to normal blood pressure. The proposed adhesion method indicates satisfactory performance.

#### Computer-aided Simulations in the Regulatory Approval Process of Medical Devices

S141

#### INTRODUCTION TO COMPUTER-AIDED SIMULATIONS IN THE REGULATORY APPROVAL PROCESS OF MEDICAL DEVICES

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**Objectives:** The requirements for safety and efficacy of medical devices are constantly increasing leading to higher costs and time to market. Simulations are proving to be particularly promising in accelerating the approval process and at the same time further increasing patient safety. The FDA related Medical Device Innovation Consortium predicts that in some years about 40% of the evidences for regulatory approval are coming from virtual patients and simulations. What is the status today and how do we get there?

**Methods:** Simulations can improve the regulatory assessment in various phases of the product life cycle, e.g. to justify design adjustments, worst-case estimates of sizes and variants, definition of test loads for experiments, to perform virtual tests on a large number of patients/conditions or to identify the root cause of failure. This may especially be important in consideration of the future requirements of the MDR for patient-specific implants.

**Results:** So far, the use of simulations was hindered by a lack of guidance and expectations within the medical device community. However, today the FDA and ASME are strongly supporting the use of this approach with new guidelines (guidance 1807) and a V&V standard (V&V40). "FDA's Office of Science and Engineering Laboratories has committed significant resources for transforming computational modeling from a valuable scientific tool to a valuable regulatory tool because of its potential for significant cost-savings in evaluating medical devices, simulating performance under scenarios that may not be possible with human use or that could more effectively be evaluated with simulation."

**Discussion:** Computer-aided simulations have the potential to revolutionize the field of regulatory approval. However, it is crucial that the simulations are performed with care and its limitations are understood. To foster confidence and wider acceptance of in silico methods, a proper methodology is needed to ensure appropriate credibility.

S142

#### VAD-CIRCULATION INTERACTION – THE VAD'S PERSPECTIVE

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**Objectives:** In case of chronic mechanical circulatory support, it is highly desirable to fully understand the interaction between the beating left ventricle and the VAD in order to potentially reduce adverse events. Thus, we took the VAD's perspective and investigated the flow inside a VAD in an advanced development status during the time course of a heartbeat.

**Methods:** The study is conducted with computational fluid dynamics (CFD) applying realistic pressure boundary conditions for left ventricular and arterial pressure and the results are compared to results produced with an established, active mock circulation loop. Due to the high resolution in time and space and the use of sliding mesh interfaces, local flow phenomena during different phases of systole and diastole are unmasked. The effects of inertia in the pump and cannulae become evident, which are not modeled in more traditional VAD flow simulations with steady-state boundary conditions.

**Results:** The results show that hysteresis effects due to inertia are quantitatively of high importance. The hysteresis curves of the VAD operational point obtained experimentally and numerically correlate well. Moreover, the results show that a VAD design for a highly pulsed flow can lead to a situation, where almost no remaining stagnation zones, as quantified with a numerical stagnation index, exist during a heartbeat.

**Discussion:** Despite increased computational costs, highly resolved CFD simulations in time and space with realistic time-varying boundary conditions are a powerful tool to increase understanding of the interaction of the mechanically supported circulation and the device itself. This enabled a verification of a VAD design with low to almost non-existing flow stagnation at constant rotational speed. This finding correlates well with the fact that the VAD does not require any anticoagulation in chronic animal trial testing lasting weeks to months.

**Acknowledgements:** The authors thank the Erich and Hanna Klessmann foundation, Germany.

S143

#### THE USE OF SIMULATION TOOLS IN THE DEVELOPMENT PROCESS OF HEART ASSIST DEVICES

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**Objectives:** In the development process of heart assist devices, numerical simulations become more and more essential. The variety of applications comprises different types of cannulas or flow geometries of ventricular assist devices (VADs). The analysis of the flow field inside VADs by means of Computational Fluid Dynamics (CFD) is extremely useful. Numerical parameter studies allow the treatment of several device related problems in conjunction with a well-balanced cost-benefit ratio.

**Methods:** Results obtained from properly performed CFD calculations permit the determination of flow induced blood damage of new flow designs in VADs and enable the prediction of the risk of harmful hemolysis for the patient. The literature provides different models for blood cell damages. In application, a proper definition of acceptable thresholds is required, in order to optimize blood guiding geometries as rotor blades or diffusor shapes in VADs. Another application of CFD can be found in the field of flow path optimization of new cannula designs. Corresponding results can lead to various design changes and can significantly contribute to a comprehensive risk analysis for regulatory assessment.

**Results:** Based on the numerical results, geometrical design iterations can considerably improve the flow design of blood pumps and cannulas. Thoroughly optimized designs exert reduced shear stresses to the cells and create less damage to the patient's blood. Furthermore, CFD studies can provide valuable information in the process of risk analysis during the regulatory assessment of heart assist devices and support the interpretation of experimental in-vitro investigations.

**Discussion:** Although numerical simulations represent a commonly used tool during research and development in the field of heart assist devices, the introduction of strict requirements and well-defined guidelines

would considerably simplify the implementation of numerical studies for regulatory approval and enhance the comparability of independently gained results.

S144

#### DESIGNING AND TESTING VAD USING THE DIGITAL AVATAR

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**Objectives:** To analyze the hemodynamic effect of ventricular assist devices using a multi-scale mathematical model of the human extracellular fluid system.

**Methods:** A Ventricular Assist Device (VAD) model has been incorporated into the Digital Avatar, a multi-scale, closed-loop mathematical model of the human fluid system is composed of a network of major arteries, major veins, and lumped parameter models for the heart, pulmonary circulation, arterioles, capillaries, venules, cerebrospinal fluid and brain interstitial fluid. The mathematical model relies on one-dimensional PDEs for blood vessels and on ODEs for lumped parameter models.

**Results:** Based on the model, we have quantified the hemodynamics of the systemic circulation with and without the medical device in healthy and unhealthy scenarios. Results will be shown at the conference.

**Discussion:** Traditionally, bench testing, animal studies and clinical trials are used as the main source of evidence for getting medical devices on the market in the US. The associated costs and time are enormous and represent a burden for MedTech companies. In addition, the FDA wants to minimize animal and human study testing. In recent years, Computational Modelling and Simulation (CM&S) has made huge steps forward. CM&S are a valuable regulatory tool for its significant cost-savings in evaluating medical devices, simulating performance under scenarios that may not be possible with human use and quantifying the safety and efficacy of medical devices. However, the existing CM&S tools are highly specialized for each field and do not provide a platform that integrates all human body mechanisms. Computational Life's vision is to replicate all body mechanisms into a single, integrated software called Digital Avatar platform. The Digital Avatar platform will enable MedTech companies to perform feasibility testing and quantify the safety and the efficacy of (ideally) any type of medical device. Here we will show an application with the VAD system.

S145

#### HEMODYNAMIC MODELING IN INTRACRANIAL ANEURYSMS - WHY SIMULATIONS ALONE CANNOT SOLVE THE EQUATION

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Hemodynamic simulations are increasingly used to assess the flow phenomena in intracranial aneurysms (IAs). Based on detailed simulation results existing therapies such as coiling or stenting can be improved or novel approaches are tested. However, the accuracy of patient-specific blood flow acquisitions strongly depends on preliminary and highly interdisciplinary steps. These include for instance medical imaging, image reconstruction, image segmentation and acquisition of appropriate boundary conditions. To assess capabilities, but also limitations of

state-of-art IA modeling techniques, international simulation challenges are frequently organized. These comparisons address single or combined steps along the simulation process. Within this talk, the most relevant competitions are presented and the main findings are summarized. In this regard, sources of error in the context of hemodynamic modeling are revealed and the awareness for standardized simulations is sharpened.

**Acknowledgements:** The presented studies were partly funded by the Federal Ministry of Education and Research in Germany within the Forschungscampus *STIMULATE* (grant number 13GW0095A) and the German Research Foundation (grant number 399581926).

S146

### NUMERICAL SIMULATION IN THE PRODUCT LIFE CYCLE OF CUSTOM IMPLANTS

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The ongoing development of modern manufacturing processes, as well as the automation and cross-linking of production facilities opens up new freedoms for the individualized manufacturing of medical devices. The patient-specific design of these highly loaded devices is associated with a high degree of experience on the part of the designer. The assessment of the safety and performance of these “lot size 1 products” cannot be carried out economically by mechanical testing. This results in a verification gap in the product life cycle of patient-specific medical devices. A verification of the design and thus the evaluation of the function and safety of the products can only be carried out economically with the help of automated numerical simulations. A software to perform virtual strength assessment of custom implants was developed. The lecture shows how the verification process of patient-individual designs with numerical methods, can contribute to the increase of patient safety. In addition, the integration and documentation of numerical simulations within the product life cycle will be presented using industrial examples.

S147

### INSILICOTRIALS. COM: A CLOUD-BASED PLATFORM OF IN SILICO TOOLS TO SUPPORT R&D AND THE REGULATORY SUBMISSION OF NEW MEDICAL DEVICES AND DRUGS

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**Objectives:** Computational tools for accurate evaluation of healthcare products are increasingly being recognized by the European Parliament, US Congress and FDA and represent a highly valuable tool for speeding up and reducing investments associated with the demanding certification process of medical devices. InSilicoTrials Technologies is developing a collaborative, automatized cloud-based platform to assist medical device and pharma companies in performing modelling and simulation activities to accelerate medical innovation while responding to recommendations of European and US parliaments and regulatory bodies.

**Methods:** Simulations provided by scientific partners are integrated within a secured and privacy-preserved environment, ultimately providing the users (companies, research and clinical centers) with in silico solutions for different steps of the R&D process on medical devices and drugs. Simulation results as well as an automatically created simulation report can be formatted in conformity with guidelines provided by EMA and FDA while taking in consideration the new European Medical Device Regulation.

**Results:** Tools currently hosted on the platform are: NuMRIs, an automatic tool to assess magnetic resonance imaging radio-frequency safety of medical devices, implemented in collaboration with the US FDA Center for Devices and Radiological Health and ANSYS. QT/TdP Risk Screen, an automated tool to assess drug-induced pro-arrhythmic and Torsade de Pointes risk of small molecules, developed in collaboration with Universitat Politècnica de València and Fundació Institut Mar d'Investigacions Mèdiques.

**Discussion:** The use of computational technologies to support medication registration is limited in scope or absent while the agencies are promoting the use of M&S in R&D and registration. This causes a gap in know-how between reality of the industry and regulatory expectations. InSilicoTrials bridges these gaps and helps industries to conform to regulatory recommendations in a timely and cost-effective manner.

### Dynamics of Albumin Conformation and its Behavior

S151

### ALBUMIN PERMEABLE MEMBRANES IN HEMODIALYSIS - A CONTROVERSY REVISITED

J. Vienken\*<sup>1</sup><sup>1</sup>Vienken, Vienken Consultant, Usingen, Germany

**Objectives:** Current understanding of membrane performance in hemodialysis implies that their permeability in terms of molecular weight cut-off must be restricted to a figure below the molecular weight of albumin, i.e. < 60.000. Nephrologists commonly request a maximal allowable albumin loss during a 4h treatment to be below 4g per session.

**Methods:** Recent investigations have shown that the 3D-conformation of albumin is unstable and changes in e.g., uremia or liver failure. Thus, detoxification efficiency of albumin may decrease by about 70% and can be explained by a considerable reduction of albumin's binding constants for both pharmaceutical drugs and uremic retention solutes, as well as for exogenous toxins. As a result, free concentrations of such solutes increase in patient's blood and a higher toxicity is observed. Binding constants for defined uremic retention solutes, such as p-Cresylsulfate, are further affected by the simultaneous presence of other solutes, such as Indoxylsulfate.

**Results:** Oxidative stress, as manifested in many dialysis patients, further promotes changes in albumin conformation. It's thus no surprise, that hemodialysis with protein-permeable membranes improves patient performance, as shown recently in a clinical trial by Nagai et al., (Ther Apher Dial, 2017; 21:378). They reported, that in a 7-year observation period, albumin leakage of 3g or more per HD session provided a better prognosis than albumin leakage less than 3g. Obviously, a clinically acceptable large albumin leakage provides beneficial effects on maintenance HD patients.

**Discussion:** The performance of albumin as an adsorbing protein is inefficient during uremia and liver failure. Due to lower binding constants for pharma-drugs and uremic toxins following conformational changes in the sick state, albumin is unable to perform like in the healthy state. It should, thus, be removed. Identification of 3D-changes of albumin and thus its removal should become a priority.

S152

### DO STABILIZERS INFLUENCE THE CONFORMATION OF THE ALBUMIN MOLECULE?

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**Objectives:** Human serum albumin (HSA), one of the dominating proteins in human plasma with its exceptional binding capacity plays an important role in storing and transporting different substances throughout the human circulatory system. Clinically used to treat a variety of diseases, particularly in liver failure therapy, pharmaceutical-grade HSA contains high amounts of stabilizers, such as Na-caprylate and N-acetyltryptophanate. These compounds represent potential risks to patients with restricted liver function. The aim of this study was to test if the elimination of stabilizers by adsorbent technique affects HSA's secondary structure.

**Methods:** Albumin infusion solutions (BIOMED, Germany) were filtered using a commercial system Hepalbin (Albutec, Germany). Raman- and FTIR spectroscopy as well as DSC analysis were applied to analyze conformational changes in treated and untreated HSA samples. For Raman based studies different parameters such as object slides type, integration time, accumulations number and laser capacity were investigated.

**Results:** Preliminary results of the physical-chemical analyses showed some differences for filtered samples in comparison to the unfiltered ones. Raman and FTIR spectra exhibited both, peak shifts in characteristic HSA amide bands and an alteration in the peaks' shape. DSC thermograms revealed significant differences in denaturation temperatures of the compared samples.

**Discussion:** A Raman shift in amide I band and changes in the  $\alpha$ -helix/ $\beta$ -sheet ratio detected via FTIR spectroscopy indicated a possible alteration in the HSA conformation after filtration. These findings were corroborated by DSC showing a tendency towards lower denaturation temperatures in samples after stabilizers removal. The results may contribute to finding a compromise between stability and high functional performance of HSA for the improvement of liver failure therapy.

**Acknowledgements:** The research was supported by members of ALBUNET e.V. expert group.

S153

#### ALBUMIN TRANSPORT FUNCTION IN PATIENT AND COMMERCIAL ALBUMIN PREPARATIONS

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**Objectives:** Albumin is a blood protein with various physiological functions as there are: maintenance of osmotic pressure, antioxidant and transport function for endogenous and exogenous ligands. Binding of these ligands can affect conformational changes of human serum albumin.

**Methods:** The "Albumin-functionality-test" is based on EPR spectroscopy and detects modified binding and functional characteristics. The used radical supporting spin probe binds analogous ligands variably strong at different albumin binding sites and thus, serves as an indirect marker for serum albumin functionality with regard to its ability for binding, transport and detoxification. A comparison of three different mixtures of albumin and a spin-labeled fatty acid allows for the *in vitro* simulation of binding-, transport- and release-conditions by the *Albumin-functionality-test*. Thus, an "effective" albumin concentration could be determined. It quantifies the amount of functional albumin in the patient in comparison to healthy controls.

**Results:** We applied Albumin-functionality-test to albumin preparations differing in redox state of cysteine-34, namely human mercaptalbumin (HMA), human non-mercaptalbumin1 (HNA1) and human non-mercaptalbumin2 (HNA2) and found differences in their functionality. The analysis of samples from patients with different states of liver failure showed a strong correlation of binding efficiency and detoxification efficiency, depending on the severity of the disease.

**Discussion:** Due to the increasing numbers of studies using commercial albumin preparations for the treatment of patients with liver disease, we investigated different available albumin samples regarding their albumin functionality. We found dramatically reduced binding and detoxification efficiencies. Our findings might be used for patient selection and prognoses for personalized therapies. Taken together, these results might affect the importance of albumin from the patient view and refer to its stability as a drug.

S154

#### ALBUMIN-BINDING CAPACITY AND DRUG PHARMACOKINETICS IN INTENSIVE CARE

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**Objectives:** The use of loop diuretics like furosemide is discussed in intensive care medicine regarding their clinical efficacy and safety. The hypothesis arose whether both parameters depend on the capability of serum albumin to transfer the drug to the kidneys.

**Methods:** Serial blood & urine samples were taken from intensive care patients receiving furosemide infusions for volume overload. Free furosemide serum fractions & urine concentrations were determined by HPLC/LCMS. Albumin binding capacity (ABiC) was quantified by adding a binding site II (diazepam binding site) specific fluorescence marker (Dansylsarcosine, DS). The amount of unbound DS was detected by fluorescence analysis after ultrafiltration, corresponding with ABiC.

**Results:** 62 patients were assessed. 50 patients (30male, 67,5 +/- 10,3 years) were included into statistical analysis. Main reasons for intensive care admission were sepsis (n=18) and perioperative care. Indications for furosemide were volume overload in acute or acute on chronic kidney injury (n=42), volume management in sepsis, ARDS or lung edema (n=8). ABiC was reduced to 65,2 +/- 12,2%, The free furosemide fraction (normal <5%) correlated negatively with ABiC (r -0,638, p<0,001) with 15,76 +/- 11,52% in those with ABiC<60% and 5,36 +/- 5,29% in ABiC 60% (p<0,001). The urinary fraction of furosemide (normal >65%) was significantly lower in those with ABiC <60% as compared to 60% (7,47 +/- 5,83% vs. 23,98 +/- 17,91, p<0,01). Furosemide induced increase in urine output was strongly correlated with ABiC (r=0,908, p<0,017).

**Discussion:** Our results indicate a role of ABiC in the pharmacokinetics of furosemide, impacting its free serum fraction and urinary fraction. Data are suggestive for a link between status of the albumin-dependent drug carrier mechanism and the safety and efficacy of the drug. Clinicians will profit from our results regarding safe use of loop diuretics & furosemide responsiveness in patients with volume overload and kidney injury.

S155

#### PERFORMANCE OF THE SECONDARY CIRCUIT IN ALBUMIN DIALYSIS EXEMPLIFIED BY THE ADVOS SYSTEM

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**Objectives:** ADVOS multi is a recirculating albumin-based dialysis device that supports kidney, liver and lung function by eliminating CO<sub>2</sub>,

water-soluble and protein-bound substances. In the present work in vitro data on the removal of these substances and CO<sub>2</sub> are presented.

**Methods:** An ex vivo model using porcine blood was established and applied in detoxification tests for water soluble and protein bound retention solutes. 3 x 3.3L of blood with high bilirubin (30 mg/dl) and lactate levels (>10 mmol/l) were treated with ADVOS multi for 4 hours each. This design, with 3 phases changing blood every 4 hours, led to high concentrations of both markers in blood during the 12 h test period. For CO<sub>2</sub> removal tests, 5 liters of blood were used instead. In both cases, a continuous CO<sub>2</sub> supply through an additional dialyzer was applied.

**Results:** Bilirubin and lactate were efficiently removed during 12hrs of in vitro detoxification. Lactate removal rates were 90%, 86% and 84% for phase 1, 2 and 3, respectively. Bilirubin elimination rates were 66%, 62%, and 57%, resulting in a total elimination of 1150 mg in 12 hrs. Albumin binding capacity was determined to be >76% at the end of the treatment indicating that albumin was not denatured in the dialysate of the ADVOS system. CO<sub>2</sub> removal with ADVOS multi depends on three variables: 1) The amount of supplied CO<sub>2</sub> depends on concentrate flow affecting both, blood pCO<sub>2</sub> and bicarbonate levels; 2) blood flow, and 3) dialysate pH and composition (i.e., carbonate concentration). A maximum CO<sub>2</sub> removal of 142 ml/min was achieved with a carbonate-free dialysate at pH 10, a blood flow of 400 ml/min and a concentrate flow of 160 ml/min. Given that blood gases are maintained within physiological conditions, a CO<sub>2</sub> removal rate of 61 ml/min can be achieved. During all the experiments blood pH was set to 7.35-7.45.

**Discussion:** ADVOS multi is a device using albumin recirculation in its secondary circuit. Here, albumin binding capacity remains stable through a systematic modification of its tertiary structure through temperature and pH changes in the ADVOS multi circuit. This facilitates the release of toxins from albumin and allows for further binding. Moreover, presence of albumin, variable dialysate composition and the flexible dialysate pH might facilitate the treatment of patients with multiple organ failure.

S156

### SOLVENT REGULATION OF HUMAN SERUM ALBUMIN PROPERTIES

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**Objectives:** To elucidate the role of the solvent and pH in the conformational changes of human serum albumin (HSA) and its binding to fatty acids. Albumin is a key biomolecule, found in most fluids of the body. HSA functions as depot and carrier for many compounds like fatty acids and affects the pharmacokinetics of drugs. Importantly, HSA acts as a toxic waste handler, displays pseudo enzymatic properties and it is a valuable biomarker in many diseases. Structurally, HSA is a very soluble, extremely robust and usually monomeric protein, which features three  $\alpha$ -helical homologous domains that fold into a characteristic heart shape. Despite its importance, the reversible pH-dependent conformational transitions of HSA in solution are not yet fully understood.

**Methods:** To compute the stability differences between the different conformers of HSA, we combine molecular dynamics simulations (MD) with free energy calculations. We determine the conformational free-energy change between conformers of HSA obtained upon introducing perturbations in the surroundings (pH, solvent mixtures) and its physiologically native state. Extended constant-pH MDs followed by Gaussian

accelerated molecular dynamics simulations allow us modelling these systems at different pH values. We perform free energy perturbation calculations to characterize the binding sites of fatty acids to HSA under different conditions.

**Results and Discussion:** We elucidate, at the molecular level, the effects of the solvent and pH on the conformational transitions and functionality of HSA. Our work allows establishing ways to modulate such transitions by tuning key biomolecular interactions, with implications for biomedical research.

**Acknowledgements:** This work is supported by the German Research Foundation (DFG) under Germany's Excellence Strategy – EXC-2033 – ID: 390677874. The Gauss Centre for Supercomputing e.V. (www.gauss-centre.eu) provided computing time on the GCS Supercomputer JUWELS at Jülich Supercomputing Centre (JSC).

### Tissue Engineered Vascular Grafts for Access in Hemodialysis

S161

### VASCULAR ACCESS IS A KEY ISSUE FOR SURVIVAL FOR UREMIC PATIENTS

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Vascular access is a key issue for survival for uremic patients that need acute or chronic dialysis.

In acute settings the use of central venous catheters, holding two luminae, are most commonly used in the intensive care for continuous or intermittent hemodialysis. Such catheters may be of acute type or a chronic type containing a separate cuff to limit bacterial invasion into the vessels. Another option is to place a peritoneal dialysis catheter into the abdominal cavity. If using the right technique, this catheter can be surgically inserted in local anesthesia and could be used directly postoperatively for acute but also chronic peritoneal dialysis.

For chronic hemodialysis (HD) the preferable option is to place an arteriovenous fistula (AVF) at the lower arm. This results in enlargement of the vein, and partly fibrosis, that allows for frequent punctures leaving no material externalized after the end of a HD procedure. A problem is the development of vascular stenosis and thrombosis that need interventions in at least 50% of patients within one year. When the AVF fails an option is to insert a graft (AVG). This may be either a biologic or synthetic graft interpositioned between the feeding artery and a more proximal vein below the elbow instead of the local vein that usually is occluded.

If this option even fails, the patient has to rely on a sufficient permanent central dialysis catheter where complication such as bacterial contamination and occlusions are problems.

To avoid such catheters a dream for future would be a graft as AV access that is made of an autologous biodegradable material that has physiological characteristics that prevent stenosis and clotting and also is possible to easy correct in its diameter to adjust for the volume of blood returned/minute to the heart to avoid congestive heart failure.

S162

### TISSUE ENGINEERED VASCULAR GRAFTS FOR ACCESS IN HEMODIALYSIS

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**Objectives:** End-stage renal disease is increasing dramatically in the western world and represents a major challenge for health care. Due to organ shortage transplantation can help only a few patients who otherwise require lifelong hemodialysis. Access for hemodialysis is an essential point and carries a high morbidity mainly due to occlusion of arterio-venous fistulas (AVF) and/or grafts (AVG). Therefore, alternative graft material has to be considered such as tissue engineered vascular grafts for access in hemodialysis.

**Methods:** Besides patient characteristics and technical issues of surgical procedures the choice of the vascular graft is essential. Despite optimization over the last years, the clinical results are still sub-optimal due to early thrombosis and late intimal hyperplasia of the non-degradable synthetic vascular grafts such as Expanded-polytetrafluoroethylene (ePTFE), or, polyethylene terephthalate (Dacron).

**Results:** New material developments and tissue engineering techniques have shown good pre-clinical and initial clinical results for AVG implantation in hemodialysis patients. These range from acellular and cell-based, stable or bio-degradable, synthetic scaffolds to biological (mandril-induced) or decellularized grafts as well as self-assembly technologies.

**Discussion:** This symposium will review the clinical need and especially address new developments of vascular grafts based on tissue engineering.

S163

#### EU REGULATIONS AND THEIR IMPACT ON BIOENGINEERED VASCULAR GRAFTS

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Extracorporeal therapies, such as hemodialysis, apheresis or blood oxygenation, are only possible when recurrent blood access is feasible. Both, artificial grafts, e.g. made of polyethylene-terephthalate (Dacron®) or a fistula are not ideal models for that purpose and are commonly considered to represent the Achilles' Heel of extracorporeal blood circuits. A stable, implantable biologic vascular graft would be an ideal solution for the benefit of patients and many bioengineers are working on this dream to come true. When considering such a vascular graft to be used clinically and as a product to be sold in the medical device market, a series of problems with regard to approval have to be solved and some fundamental questions be answered. Regarding approval, current EU regulations refer to either the *Medical Device Regulation (MDR)* from 2017 or to *Advanced Therapy Medicinal Products Regulation (ATMPs)* from 2007. Under the premise "Better safe than sorry!" regulations, such as MDR and ATMP, represent a European law. A bioengineered vascular graft is taken as a "combination product", as it is commonly made of biological cells and a (possibly) biodegradable scaffold material. When implanted, it would represent a high-risk class III medical device under the MDR regulation and would require a CE-mark. The ATMP regulation, however, says that a combination product has to be approved following the "principal mode of action". For products containing viable cells or tissues, a specific approach has been taken by the EU. For these products it holds true that "whatever the role of the medical device, the pharmacological, immunological or metabolic action of these cells are considered to be the principal mode of action". Under these premises, a combination product, such as a bioengineered vascular graft, is regulated under the ATMP rules, which renders approval rather complex. Consequences refer to the need of Phase I-III clinical trials including post-market surveillance

S164

#### IN VIVO TISSUE ENGINEERED VASCULAR GRAFTS ADAPTABLE TO AV SHUNTS

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We have developed autologous *in vivo* tissue engineered vascular grafts (*in vivo* TEVG, i.e. BIOTUBE) mainly composed of fibroblasts and collagen constructed in the patients' subcutaneous tissues. After long term animal experiments over 5 years, we clinically applied them to the congenital heart surgery of a 2-year-old child as a pulmonary artery patch plasty material in 2015. Three years after implantation, 3D-CT exhibited neither aneurysmal dilatation, significant calcification, nor shrinkage of the patch graft interfering the growth of the pulmonary artery. The clinical course was good without any significant complications over 4 years presently. However, since the tissue formation largely depends on the regenerative ability of the host, individual differences exist, and it is an issue to secure the mechanical reliability of the grafts formed in high-risk patients. We are currently evaluating the changes in mechanical properties of TEVGs by chemical treatment, and are examining whether it is possible to improve mechanical durability. In addition, in order to fabricate the TEVGs in healthy individuals as allogeneic (parents) or xenogeneic tissue substitutes, we are conducting transplantation experiments on decellularized tissues. The *in vivo* TEVGs could also be applied to AV-shunts. However, in the animal pilot experiments, significant stenosis was observed at the anastomosis with host vein resulting in early occlusion probably because of high shear stresses. The attempts to overcome the problems by several investigators are also reviewed.

S165

#### ECM HYDROGEL FROM HUMAN PLACENTA FOR SURFACE COATING AND VASCULAR TISSUE REGENERATION

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**Objectives:** Human placenta tissue has great advantages to be used as tissue source due to its molecular composition and its availability as clinical waste product. In our previous studies, human placenta arteries were decellularized and showed low immunogenicity and a supportive environment for cell migration and proliferation. Decellularized extracellular matrix (ECM) hydrogels are a widely-used tool in the field of tissue engineering and regenerative medicine. To create placental ECM hydrogel (pECM-HG) decellularized arterial grafts were further processed by an enzymatic pepsin digestion.

**Methods:** pECM-HG were biochemically analyzed. Scanning electron microscopy was used to visualize fiber structures. Cell adherence and migration assays were carried out on pECM-HG coated culture plates to confirm cytocompatibility of the material. As next step pECM-HG was used for coating of vascular grafts in a reseeding experiment using a perfused 3D bioreactor system. Furthermore, biomechanical properties of 3D hydrogels were tested by rheological measurements.

**Results:** pECM-HG showed low amount of DNA residuals and preservation of cell interacting ECM proteins. SEM analysis revealed the formation of specific fibrous structures, dependent on respective hydrogel concentration. Cell binding and viability assays showed significantly enhanced cell adherence and increased proliferation rates when seeded on surfaces coated with pECM-HG ( $p < 0.001$ ) compared to controls. pECM-HG showed beneficial properties for cell reseeding on acellular vascular grafts in a flow bioreactor system.

**Discussion:** We developed a hydrogel from human vascular tissue that is cytocompatible and supports cell adherence and proliferation. We hypothesize that this hydrogel has great potential due to its origin and composition. Further investigations on the hydrogel composition are ongoing. It is planned use the pECM-HG for 3D cell printing applications or as injectable hydrogel to support ischemic injury regeneration.

### Regulatory Aspect of Biomedical Restoration of Organ Functions

S172

#### FROM MDD TO MDR – THE VERY SHORT TRANSITION TO THE MEDICAL DEVICE REGULATION

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The new European Medical Device Regulation (MDR) is applicable on 26th May 2020. Then the current Medical Device Directive (MDD) and its national transpositions like the current German Medical Device Act (Medizinproduktegesetz) will be replaced by the new rules. Further transitions periods for existing certificates will not help for devices of the lowest risk class and will not free parties from taking into consideration the new rules for handling of device problems or registration. The new certification of existing devices in time will be challenging, because the accreditation and notification of notified bodies in accordance with the new requirements, which are needed for certification of higher risk class medical devices, is ongoing very slowly. The new European database is still not gone live, but the device and economic operator registration is dependent on the functioning of that database. A strong focus of the new regulation is on clinical evaluation of medical devices, which will leave manufacturers and developers probably with open questions. For example, what extend of clinical data is sufficient for keeping an existing device on the market? The presentation will highlight several obvious or less obvious changes due to the new regulation. It will provide guidance where to find additional answers for specific questions. And it will give an outlook on upcoming additional legislation and guidance.

S173

#### REGULATORY PROCEDURES FOR OPHTHALMIC DEVICES AND IMPLANTS

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Cataract surgery followed by implantation of an intraocular lens (IOL) is one of the most frequently performed surgical procedures in medicine. In 2020 alone, there are expected to be 32 million interventions. Here, ophthalmologists can look back on 70 years of experience. Since there is

no blood perfusion at the lens, however, the IOL has a comparatively simple immunological special position.

Furthermore, the ophthalmologists also have extensive experience with implants in the cornea, in the chamber angle of the eye and on the retina.

In the presentation an overview will be given of the current implants and the necessary regulatory processes. In addition, own experiences (with medical devices) with the new requirements of the MDR will be described.

S174

#### TISSUE IMPLANTS AND THEIR DERIVATIVES

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The generation of complex cellular structures for human application often requires a matrix, defining the three-dimensional shape and mechanical properties of the artificial tissue. The matrix as such is not viable. If this matrix is of human origin, Directive 2004/23/EC (in its national version) shall apply. In that case only "non-substantial" processing methods may be utilized, which are specified in Regulation 1294/2007/EC. For example, non-cellular substances can be extracted from the tissue, so-called derivatives. These derivatives, if of human origin, are also tissues within the meaning of Directive 2004/23/EC. If these derivatives are further processed, or if the tissue originates from animals, the matrix is classified as a medical device. The Human Tissue Authority describes the delimitation as follows: "Derivatives of non-viable tissues and cells such collagen fillers (i.e. collagen extracted from tissues and cells) fall under the Medical Device Regulation (MDR). Non-viable tissue and cell products such as demineralized bone matrix, or acellular human tissues or tissue matrices, will not be covered by the MDR. They will continue to fall under the Directive 2004/23/EC."

The paper deals in more detail with the problem of demarcation and describes the consequences of this in practice.

We will discuss the problem of demarcation in more detail as well as describe the consequences in practical implementation.

#### Novel Methods in Cryotechnology

S181

#### ALGINATE ENCAPSULATION FOR STORAGE AND TRANSPORT OF MESENCHYMAL STROMAL CELLS AT POSITIVE TEMPERATURES

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**Objectives:** Development of simple and effective technologies for cell storage at positive temperatures facilitates to extend their use and ensures safe transport between research and clinical centers. The aim is to study the viability and metabolic activity of human mesenchymal stromal cells (MSCs) encapsulated in alginate microspheres (AMS) during storage at positive temperatures.

**Methods:** The experiments were performed on human dermal MSCs derived from adult donors after their informed consent. MSCs in suspension and in AMS were stored in sealed cryovials at 4, 22 and 37 ° C. Cell

viability and metabolic activity were evaluated by MTT- and AlamarBlue-tests and the ability to adhere to the culture plastic. Mitochondrial membrane potential was assessed with fluorescent dye JC-1. Differentiation potential was evaluated after cells induction to osteogenic and adipogenic lineages.

**Results:** Encapsulation in AMS supported viability and metabolic activity of MSCs during storage in culture medium at ambient temperature at list for 3 days. Storage of MSCs under hypothermic conditions (4°C) using special media contributed to a longer sustainability of cell viability. During storage alginate encapsulated cells retained the ability to multi-lineage differentiation, but had lower metabolic activity and mitochondrial membrane potential than cells in monolayer. On release from alginate microspheres, the cells were shown to attach, reverse metabolic activity and proliferate in a similar manner to that seen before encapsulation.

**Discussion:** Encapsulation in alginate microspheres may be considered as a cheap and robust alternative to cryopreservation for the short-term storage and transport of cells for clinical and research projects.

S182

### STRUCTURE-PRESERVING CRYOPRESERVATION OF VIABLE CELLS AND TISSUES

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**Objectives:** Current standards in cryopreservation for biomedical technology are still based on slow freezing processes inducing crystallization within the sample. In preparation for this cryopreservation regime, cells and tissues are detached from growth surfaces and dissociated into single cell suspensions. Existing infrastructures like freezing devices, lab ware, and liquid nitrogen tanks for long-term storage, are designed for the handling of suspended cells and tissues and for stock keeping purposes, these procedures usually are efficient enough. Through the addition of cryoprotective agents, the sample can cope with the emerging damaging mechanism resulting from ice crystal formation. However, diagnostically and therapeutically relevant cell systems, e.g. human induced pluripotent stem cells (hiPSCs) or their derivatives like neuronal cells, depend on cell-cell and cell-surface interactions mediated by cytoskeletal proteins. Their structures are disordered, either due to these preparatory dissociating steps or due to crystallization, resulting in loss of functionality or cell loss in general.

**Methods:** A second cryopreservation regime, the vitrification, avoids crystallization within the sample and thus avoids the occurring damaging mechanisms (e.g. shear forces, osmotic shock). Thereby, the structure of cells and tissues is preserved, leading to viable and functional cells even in their adherent state (with cell-cell and cell-surface contacts) after thawing. However, vitrification requires a skilled handling and so far, the infrastructure has not been established to enable routine vitrification workflows for large cell numbers.

**Results and Discussion:** Our work shows the potential and capacity of vitrification on different cell types (e.g. hiPSCs, dopaminergic neurons). We demonstrate that vitrification is superior to slow freezing regarding cell loss and functionality and holds the possibility to cryopreserve scalable and ready-to-use cell products for a broad range of biomedical research.

S183

### TOWARDS PRACTICAL BIOBANKING OF IPSC-DERIVED MEGAKARYOCYTES

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**Objectives:** Severe life-threatening thrombocytopenia may be caused by exceptionally wide range of clinical conditions. Currently, donor platelet (PLT) transfusion is the only efficient clinical approach to successfully treat thrombocytopenia. However, PLT transfusion is challenged by adverse immune responses and is limited the short shelf life of donated material. Previously, we showed the feasibility to generate iPSC-derived MKs in a large scale, which can be used as an alternative to conventional PLT transfusion. However, success of this perspective therapeutic approach requires efficient biobanking technologies that would allow long-term storage and accumulation of MKs in clinically sufficient amounts. In this study, we aimed at the development of an optimal biobanking strategies for the preservation of iPSC-derived MKs.

**Methods:** Dimethyl sulfoxide (Me2SO) and propane-1,2-diol (PD) were evaluated for their cryoprotective efficiency in iPSC-derived MKs. Comprehensive phenotypic characterization of MKs before and after cryopreservation was performed by fluorescence microscopy and flow cytometry. Capability of cryopreserved MKs to form proPLTs and release functional PLTs has been tested in vitro. Transfusion into animal model has been performed to evaluate capability of MKs to release PLTs in vivo.

**Results and Discussion:** Application of developed cryopreservation procedures allowed recovery of over 80% of iPSC-derived MKs cryopreserved with Me2SO and PD. Recovered cell populations expressed typical MK markers, including CD41, CD61, and CD42a and showed high ploidy. Moreover, cryopreserved MKs showed the capability to form proPLTs and release PLTs in vitro as well as in vivo after transfusion in animal model. Present findings propose efficient clinically relevant biobanking strategies necessary for progress in MK-based regenerative and replacement approaches.

S184

### HIGH- AND SUPER-RESOLUTION FLUORESCENCE IMAGING OF LIVING CELLS UNDER CRYO-ARREST

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**Objectives:** High resolution imaging of living cells is impaired by artefacts introduced by chemical fixation and by the rapid movement of molecules during live cell imaging. We have therefore developed tools to cryo-arrest living cells on a microscope and image them by high- and super-resolution microscopy. However, the developed equipment is also applicable to investigate the process of cryopreservation.

**Methods:** We have developed two different microscope cryo-stages that allow for continuous observation on the fluorescence microscope. One stage allows for relatively slow controlled cooling (<50 °C/min) and



warming (<150 °C/min) of cells. It allows for medium exchange during cooling and warming and thus for change of concentration of cryoprotective agents. Due to the design of this microscope stage, oil immersion objectives can be used down to at least -45°C. The second stage is designed to ultra-rapidly cool cells with >10000 °C/s to temperatures <-130°C.

**Results:** We established a protocol to reversibly cryo-arrest living cells on a microscope stage. This permits to image physiological processes in the same living cells with practically unlimited acquisition time at consecutive points in time. This allowed for super-resolution imaging and microspectroscopy measurements, which were impeded at physiological temperatures, because of the dynamics of cellular molecules. The development of a device to ultra-rapidly cryo-arrest living cells on a microscope under continuous observation allowed for the investigation of cells, which have been cryo-arrested/vitrified within <10 ms.

**Discussion:** The presented cryo-fixations methods enable a more precise measurement of molecular patterns and thus a better understanding of molecular organization in cells under physiological conditions. The developed microscopy stages should however also enable high- and super-resolution fluorescence imaging of molecular processes in cells during the process of cryopreservation.

S185

#### DEVELOPMENT OF EFFICIENT CRYOPRESERVATION PROTOCOLS BASED ON BIOMIMETICS

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**Objectives:** Recent developments in imaging technology have enabled cryobiologists to reveal some mechanisms employed by nature to cope with freezing stress. The systematic study of these mechanisms may prove useful for developing ultimately novel and efficient cryopreservation protocols. In this work, we examine the potential advantages of using infrared video thermography (IVT) for monitoring latent heat release and freeze-thaw events in 3D porous collagen-hydroxyapatite scaffolds used as a model system. In this context, we present ongoing results on the identification of warm ice-nucleating agents (INA) from Hippophae rhamnoides with potential use in cryopreservation of tissue-engineered constructs (TECs).

**Methods:** The scaffolds were prepared by freeze-drying of mineralized collagen suspensions and characterized by Raman microscopy, X-ray microcomputed tomography and differential scanning calorimetry. Visualization of freezing/thawing events in scaffolds frozen with and without cryoprotective solutions based on leaf homogenate from Hippophae rhamnoides was performed using the thermography-based platform developed at the University of Thessaly.

**Results:** Scaffolds presented 3D porous architecture and characteristic RAMAN peaks of collagen type I and hydroxyapatite (HAP). Presence of HAP decreased the specific heat capacity of collagen scaffolds. The probability of ice nucleation was higher in cryoprotective solutions with scaffolds than in solutions without. The ice nucleation temperature within scaffolds using INA was determined to be around -1.5 °C.

**Discussion:** IVT proved to be a very effective approach for visualizing latent heat release and freeze-thaw events within scaffolds frozen in cell

culture plates. INA from Hippophae rhamnoides may potentially be used for prevention of supercooling in cryopreservation. The results obtained could serve as a basis for further development of efficient cryopreservation protocols for 3D TECs.

**Acknowledgements:** The work was in part funded by the DAAD, (IP@Leibniz, project 57156199).

#### 1st Joint ESAO and ESOT Symposium - The True Global Need for (Artificial) Organs

S212

##### ORGAN RECONSTRUCTION VS.

##### MIMICKING: STATE OF ART OF BIO-SCAFFOLD AND ARTIFICIAL ABDOMINAL ORGANS

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**Objectives:** In case of organ failure, several options can theoretically be considered, thanks to the progresses in medicine, materials science and biotechnology: i) transplant the same organ from a donor, ii) replace the functions of the failing organ by an artificial one, iii) repair its structure thanks to cell therapy or iv) engineer a new one in vitro before its implantation.

**Methods:** As far as abdominal organs (kidney, liver, pancreas), the history of organ replacement follows different ways that will be considered. In addition to medical and scientific hurdles, economical or ethical issues can drive the choices for the “best” solution for the patient.

**Results:** The case of pancreas is very specific since its transplantation is not the first option chosen to treat the failing organs, whereas it represents the gold standard for the liver and the kidney. In the case of liver, we still have to face the lack of purely artificial organ to treat acute liver failure or even chronic disease.

**Discussion:** This is due to the complexity of the organs and the variety of its functions, some of them remaining relatively unknown. In this case, the option of engineering new liver in vitro combining hepatic cells of different phenotypes with an adequate matrix allowing high blood perfusion appears as the most promising option. Several approaches have been proposed, starting from the “basic” use of a semi-permeable hollow fiber membrane to host the cells to the bio-printing of the whole organ. We will discuss here the pros and cons of these different strategies.

**Acknowledgements:** This work is performed in the framework of PIA RHU Ilite “Innovation in Liver Tissue Engineering” funded by ANR (ANR-16-RHUS-0005).

#### WG Heart Support - TAH - Future of Heart Failure or Failure of the Future?

S221

##### TAH -INDICATION AND OPERATIVE MANAGEMENT

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A total artificial heart is used for orthotopic replacement of the native ventricles in cases of irreversible biventricular heart failure resulting

either from chronic dilated cardiomyopathy or fulminant acute myocardial infarction. It is essential to find the right indication. There are several surgical options to perform a total artificial heart operation. To prevent therapy-failure it is essential to set the right indication for implanting a total artificial heart. In general therapy with a TAH is feasible with good postoperative results. Setting the right clinical frame is crucial for the establishment of a therapy concept.

S222

### SYNCARDIA TAH: OUTCOMES AND SURVIVAL, THE INTERMACS EXPERIENCE

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**Objectives:** Determine the outcomes and survival of patients who receive a temporary total artificial heart (TAH) as bridge to transplant or as bridge to decision by evaluating data from the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) database in the U.S.

**Methods:** Review of the INTERMACS database between 2006 and April 2017 revealed a total of 450 patients had been implanted. The data was reviewed to determine survival rates, adverse events, and competing outcomes. Risk factors for mortality were determined using hazard function analysis.

**Results:** Data from 450 patients (87% men; mean age, 50 years) were available in the INTERMACS database. The 2 most common diagnoses were dilated cardiomyopathy (50%) and ischemic cardiomyopathy (20%). Risk factors for right heart failure were present in 82% of patients. Most patients were INTERMACS Profile 1 (43%) or 2 (37%) at implantation. There were 266 patients who eventually underwent transplantation, and 162 died. Overall 3-, 6-, and 12-month actuarial survival rates were 73%, 62%, and 53%, respectively. Risk factors for death included older age ( $p = 0.001$ ), need for pre-implantation dialysis ( $p = 0.006$ ), higher creatinine ( $p = 0.008$ ) and lower albumin ( $p < 0.001$ ) levels, and implantation at a low-volume center ( $\leq 10$  TAHs;  $p < 0.001$ ). Competing-outcomes analysis showed 71% of patients in high-volume centers were alive on the device or had undergone transplantation at 12 months after TAH implantation vs 57% in low-volume centers ( $p = 0.003$ ).

**Discussion:** Patients receiving TAHs have rapidly declining cardiac function and require prompt intervention. Experienced centers have better outcomes, likely related to patient selection, timing of implantation, patient care, and device management. Organized transfer of knowledge to low-volume centers could improve outcomes

S223

### REINHEART TAH: HASSLE-FREE HEART-REPLACEMENT

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**Objectives:** Throughout the history of medical devices, highly invasive systems underwent a series of generations prior a successful

establishment as a widely accepted therapy. The only available TAH today (SynCardia TAH) is successfully saving lives but comes with a significant restriction of the quality of life (QoL) and a stigmatization of the patients. According to surgeons this is an important factor contributing to the low number of implantations today.

**Methods:** Next generation devices using an implanted drive promise a new level of QoL and are currently developed by several institutions worldwide. Reasons for the long time to market of the next TAH generation ( $>15$  years) might be found in the enormous challenges of technological aspects together with a difficult marked situation from an investors point of view. However, a higher QoL and an increasing trust in the reliability of the next TAH generations will inherently lead to a higher number of implantations, and further the acceptance of these devices towards an established therapy option.

**Results:** The approach of the ReinHeart TAH is to adopt the well-proven hydraulic features of the SynCardia TAH while improving its current limitations. The ReinHeart TAH features a passive filling during diastole, a principal that is also successfully applied by the SynCardia TAH. The simple and robust linear driving mechanism allows for a compact design, achieving fit in the vast majority of patients. Beyond that, the drive principle makes additional sensors unnecessary. The compact, smart and usability-driven design of the external components will lead to a broad acceptance by the patients.

**Discussion:** The unique features of the ReinHeart TAH elevate TAH-therapy to the next evolutionary step with regards to robust long-term application, size, ease of use and quality of life.

**Acknowledgements:** The ReinHeart TAH development is being supported by the European Union, the state of North Rhine-Westphalia (both EFRE-Program), and the Erich & Hanna Klessmann Foundation.

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### THE PRINCIPLES OF THE REALHEART TECHNOLOGY: A NOVEL PULSATILE FOUR-CHAMBER TAH

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Realheart TAH is designed to imitate the construction of the human heart in order to create a blood flow as natural as possible. Realheart TAH consists of two pumps, corresponding to the right and left side of the natural heart. It pumps blood from the left side and right side simultaneously in a synchronized manner.

Each pump of the Realheart TAH consists of an atrium and a ventricle, with a valve cylinder in between. When the valve cylinder moves towards ventricle, the blood pumps out from the ventricle creating the systolic phase. The ventricle fills with blood when the valve cylinder moves towards the atrium creating a diastolic phase. The cardiac output of the Realheart TAH depends on the stroke length of the valve cylinder and the frequency of the movement. The left and right pump regulate cardiac output independently in order to coordinate the left/right flow balance of the circulatory system.

Similar to natural heart, Realheart TAH is integrated into the circulatory system, which facilitates the uninterrupted blood flow to the atrium, when it pumps the blood out of the ventricle in pulses. The atrium is constructed with a compliance membrane to avoid blood suction from the veins during the systolic phase.

S226

### MAJOR RISKS AND MITIGATIONS WITH TOTAL ARTIFICIAL HEARTS – A BIVACOR PERSPECTIVE

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Total artificial hearts (TAH) are used to restore quality of life to biventricular heart failure patients but can also introduce significant risks. Several features were included in to the BIVACOR design to mitigate some of the major risks, such as 1) device malfunction, 2) insufficient hemocompatibility, 3) ineffective physiological interaction, and 4) inappropriate device geometry.

To achieve a durable design the BIVACOR TAH reduces mechanical wear by levitating a single rotating impeller in a magnetic field. To reduce the incidence of single fault failures, the levitation and drive systems incorporate backup features. Large clearance gaps in the blood flow path and cyclic speed variations assist hemocompatibility. Physiological interaction is improved via autonomous left/right outflow balancing, the ability to inherently adapt total outflow based on physiological conditions, and to respond to variations in filling volumes.

Initial durability testing is ongoing with no device failures after 180 days. The redundant motor and hydrodynamic backup bearing maintain hemodynamic function when failure modes are induced. Preliminary NIH, platelet activation and vWF degradation during in-vitro tests show lower levels than implantable VADs. In-vivo results (1x90day, 3x30-day) confirm benchtop observations. When operated with either pulsatile or non-pulsatile outflow up to 15 l/min, or with the hydrodynamic backup bearing, pfHb ranged between 0.5-2mg/dL. The system can balance outflows under a wide variety of physiological conditions, while inherently adjusting flows by more than 4 l/min. Finally, device weight and size allowed a good anatomical fit in human cadavers with BSA >1.4m<sup>2</sup>.

By implementing the risk mitigating design features, the BiVACOR device aims to provide patients with a durable, hemocompatible, and physiological interactive TAH with suitable anatomical fit. Successful development of this device may ultimately lead to a long-term alternative to heart transplants.

**Acknowledgements:** Research reported in this publication was supported by the NHLBI of the National Institutes of Health under award number R44HL137454. The project was supported by the Queensland Government under the Advance Queensland program.

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### THE CLEVELAND CLINIC CONTINUOUS-FLOW TOTAL ARTIFICIAL HEART, SIMPLY SMART

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**Objectives:** Heart failure (HF) is a critical healthcare issue and a primary source of cardiovascular mortality worldwide. Globally, it is estimated that up to 100,000 people are candidates for heart transplantation, but only about 5800 transplants were performed in 2016. At Cleveland Clinic, we are developing a continuous-flow total artificial heart (CFTAH)

in an attempt to provide a viable alternative to transplantation for the growing population of patients with end-stage HF.

**Methods:** The Cleveland Clinic CFTAH is a double-ended centrifugal pump with only one moving part that is designed to passively self-balance left and right circulations without active intervention. The device has no mechanical bearings to wear out and is driven by an extremely reliable 3-phase brushless, sensorless motor. The nominal external dimensions of the latest version of our adult CFTAH design to be tested in-vivo were 98.4 mm in length, 62 mm in diameter, 160 cc in volume displacement and 486 g in weight without the cable.

**Results:** Biocompatibility of the CFTAH has been demonstrated in two full-term 90-day in vivo tests, completed without the use of any anticoagulation. Since the conclusion of these tests, our team has been working on design improvements to further reduce susceptibility to thrombosis, enhance autoregulation, improve motor controller hardware, add clinically useful features to the control interface and enhance manufacturability.

**Discussion:** Our results to date suggest that a small, mechanically and electronically simple, auto-regulated total artificial heart has significant potential to provide an important clinical option for HF patients.

**Acknowledgements:** This work is supported by the United States National Institutes of Health, Kiyotaka Fukamachi, MD, PhD principle investigator.

S228

### CARMAT TAH - IS THE FUTURE ALREADY THERE?

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The electro-hydraulically actuated Carmat total artificial heart (C-TAH) is designed for biventricular replacement in patients suffering from end-stage heart failure, either as bridge to transplant or destination therapy. It generates pulsatile flow which is automatically adapted to the patient's physiological needs. The device contains bio-prosthetic blood contacting materials, allowing for low dose anti-coagulation. A multi-centre European study is currently underway to assess the safety and performance of the C-TAH, with 6-months survival as primary objective. Preliminary results will be presented from a cohort of 10 patients included in the study.

### Muscle and Tendon Tissue Engineering

S231

#### ELECTROSPUN MATERIALS FOR TENDON AND MUSCLE ENGINEERING: TOWARDS THE RECONSTRUCTION OF THE MYOTENDINOUS JUNCTION

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**Objectives:** Bioengineering of elements of the musculo-skeletal system is rich in studies concerning bone reconstruction. Among them, the culture of cells on a osteogenic electrospun scaffold under dynamic conditions is an interesting option. However, one should also consider the reconstruction of tendon and muscle finally mimicking a continuum from bone to muscle.

**Methods:** Poly-caprolactone (PCL) 10% was electrospun on a rotating collector whose velocity varied to get random or aligned nanofibers. Bone marrow stem cells (BMSCs) and C2C12 cells were respectively seeded on the materials. Mechanical or electrical stresses were applied to the biohybrid scaffolds to differentiate cells towards tendon's or muscle's phenotypes, in the absence any specific biochemical factors.

**Results:** After 2 weeks of culture under dynamic stretching (1Hz, 4% strain for an hour followed by 11h of rest), BMSCs were found to align in the stretching direction, presented increased levels of tenomodulin and neosynthesized collagen, compared to static conditions. C2C12 differentiation until aligned myotubes stage was more difficult to achieve. We proposed to add a PEG microstructure to guide cell growth. Combined to electrical stimulation, it led to the cells fusion into very long myotubes hosting many nuclei, as expected.

**Discussion:** In this study, we managed to grow two different types of cells and generate their differentiation towards tendon and muscle lineage, in the absence of any differentiation factors. The next step is to implement the co-culture on different areas of the same electrospun scaffold, to let the cells proliferate and study the events occurring at the junction.

**Acknowledgements:** This work was by Labex MS2T (ANR-11-IDEX-0004-02) and by Région Hauts de France / FEDER (INTIM project).

S232

### HIGH-RESOLUTION PRINTING OF 3D COLLAGEN BASED COMPOSITE SCAFFOLDS FOR IMPROVED DENSITY AND STRUCTURAL OSTEO-IMPLANTS

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**Objectives:** This work aimed to print high-resolution, collagen-based, constructs via suspended 3D printing with load-bearing and compositions closer to native bone; for potential use as implant materials.

**Methods:** Collagen type I (Col) and gelatin methacrylate (GelMA) blends were systematically investigated as bio-inks, probing their rheological properties and crosslinking efficiency for printing. An adapted 3D bioprinter (3DDiscovery, regenHU, Switzerland) based on an extrusion principle was used to print constructs. Calcification was investigated, *in vitro*, using a polymer-induced liquid precursor for the mineralization process.

**Results:** Careful control over the formulation and processing resulted in refined construct properties such as: wall width (500µm), lattice length (2cm) and shape (bone trabeculae). Once printed, the ability to cure the GelMA/Col blends was dependent on photo-polymerisation methodology, with enhanced curing and lower remaining soluble fractions (10% vs 40%) for visible light + Riboflavin/SPS in comparison to UV + Irgacure. Control over the construct structure allowed defined mineralisation, and subsequent material responses.

**Discussion:** In recent years the development of 3D printing technologies has attempted to combat the growing need for bone repair solutions, although is limited by the number of bio-inks, and printable resolutions available. Suspended manufacture has sought to address this issue, using a fluid gel to support a secondary biologically relevant bio-ink whilst it undergoes a curing step, during or post-printing. To date, printing techniques have not been shown to provide fully resorbable and/or mechanically satisfactory bone implants. This research has shown

promise as the first steps towards printing high resolution constructs with chemical compositions more closely matching that of natural bone. Further works involve deeper investigation of calcification and impact on implants mechanical properties and microstructure.

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### POLYCAPROLACTONE:CHITOSAN-GRAFT-POLYCAPROLACTONE BLENDS ENHANCE BIOCOMPATIBILITY IN ELECTROSPUN SCAFFOLDS FOR TENDON REPLACEMENT

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**Objectives:** Biodegradable PCL-based electrospun scaffolds are often used in TE. However, such scaffolds lack biocompatibility, wettability and chemical accessibility for tendon replacement applications. Here, we propose a new method to overcome those shortcomings by using PCL:Chitosan-graft-PCL (PCL:CS-g-PCL) blends instead of post-process coatings.

**Methods:** Blended solutions of PCL (175 mg/ml in 2,2,2-TFE) and CS-g-PCL (1200 mg/ml in chloroform) were electrospun into scaffolds with CS-g-PCL from 0 to 90 wt% (10 ratios). SEM based measurements were carried out to investigate fiber diameter and morphology. Chitosan amino groups within the fibers were quantified by nuclear magnetic resonance spectroscopy (<sup>1</sup>H-NMR) and x-ray photoelectron spectroscopy (XPS). DSC measurements were conducted to assess thermal properties and crystallinity. Subsequently, metabolic activity of human mesenchymal stromal cells (hMSC) seeded on the scaffolds, was determined over 7 days.

**Results:** Blends with CS-g-PCL contents < 90 wt% led to fiber diameter of  $1 \pm 0.35 \mu\text{m}$  and to  $2 \pm 0.38 \mu\text{m}$  for 90 wt%. XPS measurements showed an enrichment of chitosan amino groups at the surface of the fibers. The enthalpy of fusion increased by up to 40 J/g for CS-g-PCL contents from 0 to 90 wt%, indicating a significant increase in crystallinity. hMSCs had maximum metabolic activity on samples with CS-g-PCL contents between 10 and 30 wt%.

**Discussion:** Electrospinning of blended PCL:CS-g-PCL solutions resulted in higher crystallinity while preserving mechanical properties. Furthermore, increased wettability improved biocompatibility and chemical accessibility, which ultimately influenced the metabolic activity of the hMSCs positively. This promising novel approach will increase the biocompatibility of PCL-based scaffolds with simultaneous reduction in production complexity.

**Acknowledgements:** The research project is supported by the DFG in the framework of the Research Unit 2180 "Graded Implants for Tendon-Bone Junctions".

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### ENGINEERING 3D MICROPHYSIOLOGICAL SKELETAL MUSCLE TISSUES: FROM MICROTISSUES TO VOLUMETRIC CONSTRUCTS

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**Objectives:** Engineered skeletal muscle tissues in three-dimensional (3D) cell culture platforms that resemble the muscles complex native structure and organization can be used as *in vitro* models to study muscle physiology and metabolism. This project allows us to develop a new platform to model muscle diseases (such as myotonic dystrophy 1) *in vitro* in order to study its response to candidate therapeutics and to better understand disease mechanisms of pathogenesis. To this end, we monitor the secretion of disease-associated biomarker proteins and metabolites.

**Methods:** Here, we present 3D skeletal muscle constructs, fabricated by encapsulating C2C12 cells and patient-derived transdifferentiated skin fibroblasts in a photocrosslinkable Gelatin Methacrylate and Carbolyethylcellulose Methacrylate (GelMA:CMCMA) hydrogel and cryogel scaffold. These scaffolds present a microgrooved topography that promotes cell alignment and differentiation. Electrical stimulation (ES) was then applied to the engineered tissues during cell culture to induce spontaneous contraction and maturation of the sarcomeres. Cell alignment differentiation, and the effect of ES were assessed by calculating the orientation angle and fusion index of immunostained myotubes expressing Myosin Heavy Chain (MHC).

**Results:** We have obtained a new platform to study the evolution of congenital muscle diseases, specifically myotonic dystrophy 1 and evaluate the functional tissues by metabolic and gene expression analysis. Monitor the secretion of biomarkers proteins, metabolites, and the glycolysis pathway of muscle tissues for different drug candidates.

**Discussion:** This platform has been tested with different drugs assays and represent a step toward the goal of producing *in vitro* drug testing systems for medical and pharmaceutical industry applications. Finally, such “muscle tissue-on-a-chip” devices can be fabricated using patient’s own cells as a major step toward personalized medicine.

S235

#### MUSCULAR DISEASES: CONSTRUCTION OF SKELETAL MUSCLE-ON-A-CHIP FOR NEW TREATMENTS

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**Objectives:** For 30 years, the mortality rate of patients hospitalized in intensive care unit has been drastically reduced. But an increase in muscle dysfunctions at the end of intensive care stay, leading to long term functional disability was observed at the same time. The physiological mechanism remains poorly understood due to a lack of study tools. The objective of this work is therefore to create a new tool for the tissue construction of an *in vitro* skeletal muscle. This tool should allow a muscle construction which mimics physiological reality, in order to model the disease more accurately. Also, it should allow mechanical and electrical stimulation in order to simulate the resumption of muscle contraction of patients.

**Methods:** Using sol-gel process, we synthesized a new biomaterial, based on an epoxy organic-inorganic hybrid precursor (g-glycidylxypropyltrimethoxysilane). This biomaterial was deposited as a thin layer (spin-coating process) of 7µm thickness on a silicone membrane suitable to undergo mechanical stretching. The biomaterial was microstructured using the UV laser writing lithography to create a line network. This line

network was revealed with a 2-minute isopropanol bath and we obtained lines of 8µm thickness spaced of 175µm. To ensure a biological environment and a strong adhesion of cells on microstructured silicone support during mechanical stretching, we grafted silylated bioactive peptides using dip-coating process.

**Results:** Muscular stem cells which were isolated from patients’ quadriceps biopsy were seeded and, by immunofluorescence staining, we observed a growth of muscle fibers along the lines, mimicking the physiological organization of a muscle.

**Discussion:** We were able to model the first stages of a complex muscle organoid *in vitro* using a new tool manufactured by a fast, simple and reproducible process. With the mechanical and electrical stimulation of this muscle-on-a-chip, this work should allow us to better understand these muscle dysfunctions and find new treatments.

#### Novel Smart Devices in the Treatment of CKD-Patients

S241

#### TOWARDS A NEW HAEMODIAFILTRATION APPROACH: FINDING THE GOOD CONVECTION WITH NO EFFORT

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Recent evidence from randomised controlled trials (RCTs) suggests that post-dilutional haemodiafiltration (HDF) is associated with a benefit in survival. This benefit has been mainly observed in re-analyses of the primary data looking at the patients treated with the highest convection volumes (HCV-HDF). To obtain HCV-HDF, significant pressure has to be applied to the dialyser membrane and the dialysis systems are submitted to significantly stronger transmembrane pressure (TMP) constraints, frequently surpassing the limits proposed by the guidelines. Several automatized systems are proposed to avoid or overcome the alarms induced by the increases in convection and still maintain the volumes prescribed by the physician.

The present overview will

- i) Critically analyse the evidence on the effect of convection volumes on survival.
- ii) Review the automatic systems presently proposed to achieve a given level of convection.
- iii) Analyse the possible pitfalls in determining the limits established by a dialysis monitor when performing HCV-HDF.
- iv) Explore a harmonised definition of the parameters that may drive the convection volumes during a dialysis procedure with the aim to help in better defining the Best Practice Guidelines for HCV-HDF.

S242

#### TOWARDS A PORTABLE ARTIFICIAL KIDNEY – STRATEGIES FOR IMPROVED TOXIN REMOVAL AND DIALYSATE REGENERATION

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Hemodialysis is an important therapy for End Stage Renal Disease (ESRD) patients if a donor kidney is not available. During four hours of conventional therapy, three times a week, mainly small water-soluble toxins and a limited number of middle molecules are effectively removed from the

patients' blood [1]. Besides, the therapy is non-continuous, causing large fluctuations in water balance and uremic waste, potassium and phosphate. Therefore, in recent years, many developments are focussed on longer treatment times, such as, nocturnal dialysis or application of a portable or wearable artificial kidney (PAK or WAK respectively).

The continuous therapy outside the hospital requires application of membranes with long-term selectivity, fouling resistance and blood compatibility as well as demands reduced amount of water. In fact, for WAK, a small volume of spent dialysate should be continuously regenerated and reused.

In this lecture, we will discuss all these important issues, including:

- 1) Strategies for achieving endotoxin-free dialysate since the continuous recirculation of the dialysate for prolonged time demands additional efforts to avoid microbial contamination, without extra water consumption and without affecting the overall weight of the system.
- 2) Strategies for achieving dialysate regeneration and especially urea removal since its daily molar production is higher than that of other waste solutes.

**Acknowledgements:** We would like to acknowledge the financial support of:

- Health-Holland TKI project NOVAMEM (project no: LSHM16059-SGF).
- Strategic alliance program entitled: Advanced biomanufacturing, funded by the University of Twente, Utrecht University and University Medical Center Utrecht, project title: Innovative (bio)engineering membranes for improved treatment of kidney failure.

## S243

### A CONCEPT FOR SMART REAL-TIME MONITORING OF INTERMITTENT RENAL REPLACEMENT THERAPY

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**Objectives:** One of the main functions of renal replacement therapy is removal of excess products of metabolism from blood. Uremic toxins, waste products found in the blood of patients in need of renal replacement therapy (RRT), have very high blood concentrations and are harmful to the organism. Uremic toxins have been found over 100 and their classification is based on their molecular mass and linkage to proteins. EUTox, the European uremic toxin work group, has divided uremic toxins into 3 groups:

- 1) water soluble toxins with small molecular mass (MM) < 500 Da (e.g. urea);
- 2) middle size molecules, MM > 500 Da, (e.g.  $\beta$ 2-microglobulin);
- 3) protein-bound uremic toxins (e.g. indoxyl sulphate and p-cresyl sulfate).

**Methods:** TalTech and a spin-off company grown out of the university, OFT, have developed a new, real-time multicomponent (MCM)-sensor to monitor all uremic toxin groups in RRT. MCM-sensor contains miniature LED-technology to measure UV-absorbance and fluorescence. During online measurements, the sensor is connected to dialysis machine outlet with easy-to-handle connectors.

**Results:** The results from a pilot clinical study at Nephrology Department of North Estonia Medical Centre and Nephrology Department of Linköping University Hospital will be presented. The outputs of the sensor are on-line concentration patterns of small, middle and protein-bound uremic toxins in the waste dialysis and the clinical parameters of the uremic toxin removal (removal rate, total solute removal). A

telemetry application enables to follow the dialytic removal even on remote distances.

**Discussion:** A concept for smart real-time monitoring of intermittent RRT has been realized as a novel miniaturized optical sensor performing intradialytic on-line multicomponent uremic toxins monitoring in the spent dialysate.

**Acknowledgements:** The study was funded by the EU through the ERDF H2020-SMEINST-2-2017, OLDIAS2-On-line Dialysis Sensor Phase2 project and the Estonian Ministry of Ed. and Res. under IUT 19-2.

## S244

### NOT YET REACHED THE CLINIC: CONCEPTS FOR REMOVAL OF PROTEIN-BOUND UREMIC TOXINS

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**Objectives:** Patients suffering from chronic kidney disease (CKD) show an increased cardiovascular mortality and morbidity mainly due to the accumulation of protein-bound uremic retention solutes. Dialysis therapies have been optimized for the removal of low-molecular weight water-soluble and unbound uremic retention solutes, but not for protein-bound uremic retention solutes. Therefore, there is a strong need to increase the portion of unbound fractions in order to increase the clearance for these uremic toxins.

**Methods:** We developed dialysis techniques where the protein-bound uremic retention solutes are removed more efficiently under high ionic strength or high-frequent electric fields. The protein integrity of proteins and enzymatic activities were analysed.

**Results:** The protein-bound fraction of phenylacetic acid, indoxyl sulfate and p-cresyl sulfate was significantly decreased by using high ionic strength or high-frequent electric fields

**Discussion:** Although protein-bound uremic retention solutes have a major impact on the morbidity and mortality of CKD patients, the clearance of these solutes by conventional extracorporeal therapies is low until now. These uremic toxins bind to plasma proteins with a molecular weight greater than the cut-off of dialysis membranes and therefore conventional dialysis therapies do not sufficiently remove protein-bound uremic toxins. Disruption of association with protein and uremic solute is one possibility to significantly improve the removal of protein-bound uremic toxins. Therefore, we developed approaches for an increased release protein-bound uremic retention solutes from their protein-binding resulting in an increased clearance.

**Acknowledgements:** Conclusion: Improvements of therapies for CKD patients are essential. Especial the consideration of removing hydrophobic protein-bound uremic toxins via new methods like application of high-electric fields or increased ionic strength is a further progress

## EUROOoCs@ESAO

## S251

### DEVELOPMENT OF A LIVER PANCREAS ORGAN ON CHIP COCULTURE MODEL

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**Objectives:** None Alcoholic Fatty Liver disorders (NAFLD) is a complex systemic disorder because it is associated with clinical states such as obesity, insulin resistance, and type 2 diabetes thus involving both liver and pancreas. In particular, pathological pancreas (such as in diabetic patients, in non-alcoholic fatty pancreas disorders patients) led to miscontrol of insulin secretion (the insulin modulates the lipid accumulation in liver).

**Methods:** Organ on chip approaches is one way to mimic human physiology. In this paper, we will present the development of a liver, pancreas and liver pancreas co-culture model to simulate the interaction between both organs.

**Results:** The morphological analysis confirmed the rat hepatocytes and the rat Langerhans islets were cultivated successfully after the extraction for 7 days. The tissues functionality was confirmed by the production of albumin in the liver on chip models and by the insulin secretion in the pancreas biochips. The RTqPCR analysis confirmed that the pancreas on chip culture contribute to maintain high level mRNA of genes related to glucose insulin homeostasis when compared to Petri control. Then, the GLP1 drug contribute to increase the insulin metabolism in pancreas on chip. In liver pancreas co-culture, we found that the presence of pancreas islet contributed to modify the mRNA levels of glucose-insulin homeostasis related genes in the hepatocytes. It also contributed to increase the insulin production when compared to pancreas biochip control.

**Discussion:** Those results demonstrated the potential of our liver pancreas model to be upgraded to a complex disease model.

**Acknowledgements:** The project is granted by the ANR-16-RHUS-0005. We thank Prof B.J Kim, Mathieu Danoy, Yannick Tauran and Benedikt Scheidecke for their help in the project.

S252

### INTEGRATED ORGAN-ON-A-CHIP SYSTEMS: ADVANCED MICROPHYSIOLOGICAL PLATFORMS RECAPITULATING COMPLEX HUMAN TISSUE

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Drug discovery and development to date has relied on animal models, which are useful, but fail to resemble human physiology. The discovery of human induced pluripotent stem cells (hiPSC) has led to the emergence of a new paradigm of drug screening using human patient- and disease-specific organ/tissue-models. One promising approach to generate these models is by combining the hiPSC technology with microfluidic devices tailored to create microphysiological environments. Such organ-on-a-chip platforms (OoCs) or microphysiological systems combine human genetic background, in vivo-like tissue structure, physiological functionality, and “vasculature-like” perfusion.

Using microfabrication techniques, we have developed a variety of OoCs that incorporate complex human 3D tissues, such as retinal, choroidal, cardiac, pancreatic, and adipose tissue. By generating microphysiological environments the platform is capable of keeping the tissues viable and functional over multiple weeks. The OoCs generally consist of three functional components: organ-specific tissue chambers mimicking in vivo structure and microenvironment of the respective tissues; “vasculature-like” media channels enabling a precise and computationally predictable delivery of soluble compounds (nutrients, drugs, hormones); “endothelial-like” barriers protecting the tissues from shear forces while allowing diffusive transport. The small scale and accessibility for in situ analysis makes our OoCs amenable for both massive parallelization and integration into a high-content-screening approach.

To facilitate and enable the adoption of OoCs in industrial and non-specialized laboratories, we have developed technologies for automated 3D tissue generation and for the flexible plug&play connection of individual modules to multi-organ-chips. These technologies paired with the versatility of our OoCs pave the way for applications in drug development, personalized medicine, toxicity screening, and mechanistic research.

S253

### BUILDING VESSELS ON A CHIP TO MODEL GENETIC VASCULAR DISEASES USING PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

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**Objectives:** Blood vessels are integral to the maintenance of all tissues. They deliver oxygen and nutrients and remove waste. Blood vessels not only contain endothelial cells (ECs) that are in direct contact with flowing blood cells and fluids but they may also have pericytes and smooth muscle cells on the outside that stabilize and regulate their size. Defects in EC and pericyte/smooth muscle cell interaction are often implicated in many pathological conditions. Patients with a genetic disease called hereditary hemorrhagic telangiectasia (HHT) suffer from heavy and recurrent nosebleeds, due to unstable blood vessels as a result of defective interaction between ECs and pericytes.

**Methods:** Here we used human induced pluripotent stem cells (hiPSCs) as a source of patient-specific cells, as they can be derived from all individuals, including children and patients with genetic disease and subsequently differentiated towards all cells of the body. Besides, we can now use hiPSCs to derive all the component of blood vessels in large numbers and use them to re-create blood vessels on a chip to model inflammation and disease.

**Results:** HHT-hiPSC-ECs displayed no apparent functional differences using a set of standard two-dimensional (2D) assays. The ability of hiPSC-ECs to form a perfused microvascular network was next examined using three-dimensional (3D) cultures in microfluidic chips. Using these 3D vessels on a chip, we found that in contrast to 2D microvascular cultures, the ability to form 3D microvessels in microfluidic chips was strikingly compromised when HHT-hiPSC-ECs were used compared to isogenic control ECs.

**Discussion:** This patient-based hiPSC model thus serves as the first proof of principle that vascular diseases could be modeled using patient-specific hiPSCs in 3D microfluidic chips and to identify new target cells and possible pathways for therapy.

S254

### MIMICKING THE LUNG ALVEOLAR ENVIRONMENT WITH ORGANS-ON-CHIP TECHNOLOGIES

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**Objectives:** The complex tree-like architecture of the lungs that ends with tiny alveolar sacs is difficult to mimic in-vitro. The delicate and ultra-thin

alveolar barrier with its air-liquid interface is constantly exposed to the rhythmic respiratory movements. We report here about two organs-on-chip models that uniquely reproduce this environment and in which patients' cells are cultured. A first model recapitulates an array of alveoli with in-vivo dimensions using a biological stretchable membrane. The second model mimics a functional lung capillary network.

**Methods:** The key part of the lung alveolar model is a biological membrane made of collagen and elastin (CE) that is pipetted on a gold mesh, whose pores correspond to the alveolar size (about 200µm). Once the dried CE-membrane is rehydrated, primary human cells (alveolar epithelial and endothelial) can be cultured on both sides. The second model is based on the self-assembly of endothelial cells and pericytes that are confined in fibrin gel in microengineered compartments.

**Results:** A functional alveolar barrier made of primary human alveolar epithelial cells and lung endothelial cells is reported. The CE-membrane, on which the cells are cultured, is thin (a few micrometers), porous (enables the culture of cells at the air-liquid interface during several days), and stretchable. The reported lung microvasculature made of self-assembled endothelial cells and pericytes is perfusable, vasoactive (contracts in presence of phenylephrine, a vasoconstrictor) and is permeable.

**Discussion:** These advanced in-vitro models enable mimicking the lung parenchymal environment in an unprecedented way. As a result, the cultured tissues made of primary human lung cells are able to maintain organ-specific functions, such as air-blood barrier tightness and microvascular perfusability and contractility. Organs-on-chip solutions, such as those presented here, may open new possibilities for specific tissue engineering applications.

S255

#### IN-SILICO COMPUTATIONAL AND IN-VITRO ORGAN-ON-CHIP MODELS AS ENABLERS OF THE FUTURE PERSONALIZED MEDICINE

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Precision medicine emerges from integration of a number of emerging technologies and the data they produce with modern data analytics. For precision diagnostics and for predicting drug responses new computational and in-vitro models are needed from gene regulation to cellular and organ functions. Human induced pluripotent stem cells (hiPSC) derived e.g., from patient blood cells provide means to produce most cell types and thus provide means to get patient specific in-vitro models. New technologies are needed to produce tissues from these cells and to assess the cell functions in-vitro. In addition, computational in-silico models can be used to augment our understanding of the diseases or drug effects. They also provide tools to translate the in-vitro findings to clinical settings and patient populations.

We are in transition to turn our in-vitro cell culture models to body-on-chip platforms including environmental control and biophysical functional sensing. We have developed methods to assess the cellular functions based on electrophysiological sensing as well as 2D and 3D bioimaging. For example, we have developed imaging methods to assess functions of hiPSC cardiac cell with simultaneous assessment of electrophysiology such as Calcium and voltage transients as well as mechanobiology in vitro. Further, we have developed in-silico models of various cellular function including multi-cell-type neuronal networks and in-silico population models of the hiPSC cardiomyocytes. The later ones are providing us ionic machinery of hiPSC derived cardiomyocyte electrophysiology in various populations of patients. We have shown that these computational models can represent pathological patient phenotype

cells and populations of patients with specific mutations, e.g., long QT syndrome. We have also demonstrated the power of in-silico as possible pre-screening method for drug effects prior to in-vitro examinations. Moreover, our in-silico results highlight the need of careful consideration of use of hiPSC models before they can be turned from immature cell models to mature tissues in vitro.

With integration of novel engineering expertise from multimodal sensing, imaging and computational modelling, we have shown their power on studying diseases and for pre-screening of compounds. Our results demonstrate the power of combined in-vitro and in-silico methods for future precision medicine.

#### Symposium for Multidisciplinary Artificial Organs Research Translation

S261

#### ENGINEERING MODELING AND SIMULATIONS FOR MEDICAL DEVICE DESIGN DEVELOPMENT: A CLOSER LOOK TO CARDIOVASCULAR ADVANCES

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Engineering modelling and simulation have been being used in different industries for better product and process development for already many decades. Together with the developments in medical imaging, this engineering approach can now be applied to understand how different human organs function and eventually develop devices to replace them or support their correct functioning.

A digital environment for development of artificial organs and medical devices is possible with correct application of engineering modelling and simulation. These concepts enable researchers and engineers to perform design iterations rapidly and with ease. This is why the application of engineering modelling and simulation is considered as one of the very important bio-technological advancements in the recent years and is projected to be so in the close future.

In this respect; cardiology is one of the areas where this potential is being converted to valuable outputs. Virtual modelling and analysis of a representative beating human heart, patient specific heart modelling or digital prototypes of heart devices are main research interests of an interdisciplinary community of cardiologists, surgeons, engineers, and physicians.

In the scope of cardiovascular simulations; patient specific (or normalized among a group of patients) medical imaging based CFD simulations and coupled multiphysics FSI simulations are the main approaches for generating the simulation frameworks moving forward in the road to ultimate digital twins of the human heart.

This presentation is intended to give insights about various processes that lead to higher fidelity digital representations in the scope of cardiovascular simulations, including below:

- CFD model generation based on CT scans
- Non-invasive digital angiography & FFR CT (Fractional Flow Reserve)
- Post-TAVI coronary flow
- Blood flow in SIMULIA LHHM (Living Heart Human Model) with mechanical valves
- Transcatheter tissue engineered aortic valve FSI simulation
- Evolut R stent deployment in LHHM
- Mitral Valve and Mitral Clip FSI simulation



The presentation intends to give a brief introduction on the simulation concepts/approaches which can be applied to cardiovascular problems. While it intends to encourage the community to take the advantage of these concepts in cardiovascular research, it highlights the importance of correct modelling and accurate numerical simulations.

S262

### A BYPASS GRAFT DESIGN INSPIRED BY NUCLEAR ENGINEERING

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**Objectives:** Given the extent of bypass graft failure, the motivation behind this multidisciplinary project is to improve the patency of the current bypass grafts by developing a novel and optimised blood flow augmentation technique.

**Methods:** One of the most significant contributions to the improvement of haemodynamics in grafts was based on a research which showed that the 'spiral flow' is a natural phenomenon in the whole arterial system and is induced by the twisting of the left ventricle during contraction and then accentuated upon entering the aortic arch. The benefit of this flow pattern lies in removing unfavourable haemodynamic environment such as turbulence, stagnation and oscillatory shear stress, which are believed to be the main causes of intimal hyperplasia at anastomotic configurations.

**Results:** This multi-disciplinary engineering venture has resulted in a unique product which makes use of both non-planar helicity and an optimised internal ridge within the graft to achieve a significantly improved haemodynamic condition within the anastomosis (an anastomosis is a surgical connection between autologous/prosthetic grafts and veins/arteries inside the human body)

**Discussion:** This truly multidisciplinary project has integrated fluid mechanics, biomechanics and biology with cardiovascular surgery to develop a novel biomedical device, inspired by the nuclear engineering sector. The novel spiral-inducing bypass graft, nominated for this award, is the best example of how engineering techniques, tools and designs can lead to life-saving innovations that could potentially save the lives of thousands of people and save millions of pounds for the healthcare systems across the world. Such successful engineering stories are what would encourage the next generation of engineers to go beyond the traditional boundaries of engineering disciplines to make a difference.

S263

### SENSORSTIM – RE-EXPERIENCE MOBILITY

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SensorStim Neurotechnology GmbH is a start-up of the TU Berlin. The business area is medical engineering based on neurostimulation and eHealth for rehabilitation and homecare.

Our vision is to help people with limited mobility due to paralysis, spasticity or pain and to mobilize them with technical assistance. Our novel medical device enables the use of noninvasive neurostimulation for rehabilitation and therapy of people with disabilities after damage to the central nervous system - brain, spinal cord, pyramidal tract. It is mobile, easy to use and it can be used in water, too.

Our device consists of a neurostimulator combined with body sensors and can be operated via a smart App. Furthermore, it can be used for clinical and home care. After a successful market entry, we further want to support walking, cycling and aqua gymnastics with electrical stimulation.

We started in 2018 and are currently funded by grants of the Federal Ministry of Education and Research (BMBF), Investitionsbank Berlin (IBB) and private equity and development cooperations. By the end of 2019, we plan to achieve CE marking for our first product and ISO 13485 certification for our quality management system.

S264

### INNOVATIVE TAILORED IMPLANTS – THE LONG JOURNEY FROM SCIENCE TO MARKET

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One of the pillars for a more patient oriented healthcare is found in patient individualised medicine. Custom-made implants tailored to the individual's specific requirements is one of the key elements herein to obtain better outcomes. However, the production of patient individualised implants is nowadays still often related to a high rate of manual processes, causing both high cost and long delivery periods.

Technical innovations play a crucial role in introducing these patient individualised therapy options into clinical practice. Advances in medical imaging and the evolution of new production technologies (e.g. additive manufacturing) build the basis for a time and cost effective production of implants of lot size one at industrial-scale level. Combining these aspects with digitalisation leads to the potential of a new era of patient individualised implants and appropriate business models. It is crucial to systematically review and adapt the data management and data processing throughout the entire value chain in order to achieve the goal of a more patient individualised care. Different tools are used to define interfaces, actors, data types throughout the entire process and methods need to be established for the data processing itself.

A digital process chain from diagnosis to the actual delivery of a custom-made implant to the hospital including the manufacturing process is established. The underlying principles and assumptions used are exemplarily shown. The digital process chain is used to pave the way from science into a successful business model and company, in order to introduce these patient individualised implants to achieve a better patient care.

The use of technical and medical progresses in combination with digitalisation is used to obtain a new generation of patient individualised implants. The way from science to start-up within this field is presented.

S265

### REGULATORY COMPLIANCE FOR INDIVIDUALIZED MEDICAL PRODUCTS – A STARTUPS PERSPECTIVE

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Our main objective is to develop safe medical products for a minimally-invasive surgical intervention that fulfill all requirements by the new Medical Device Regulation (MDR). The secondary objective is to minimize the time to market by implementing standard conforming processes in a minimalistic way so they can be operated by only a handful of people. The twist in the story is that one of our products of our system will be individualized in the operating theater.

While basically everybody is forced to operate in a "learning by doing" mode regarding MDR compliance, we do rely on external trainings provided by notified bodies and other sources like websites, webinars, and dedicated conferences to learn more about how to fulfil regulatory

requirements. Additionally, we are partnering with our strategic investor (a manufacturer of class-III implantable active devices) to receive trainings and advice regarding regulatory affairs. However, it has to be stressed that one has to consider all parts of the MDR, especially the annexes and the applicable norms.

Here we present our technical approach and our strategy on how we aim full MDR compliance. We established a completely digital workflow for all our documents, including cryptographic signatures, version history, reviews, mainly based on open source tools. This allows us to handle the document management very swiftly. We have one person dedicated to our quality management system. The risk management is integrated as a cross-cutting-concern into all the product development processes.

The main challenge in the planning and founding phase of OtoJig GmbH was and still is to estimate what has to be done, which norms do apply, and how much effort (time and money) it is to perform the steps and create all the required documents. A difficulty is that contradicting statements between the MDR and other applicable norms are still under interpretation and public discussion.

**Acknowledgements:** OtoJig GmbH is supported by the German Federal Ministry of Education and Research (BMBF) FKZ 13GW0265A.

### Smartificial Cardiac Devices - Clinicians Meet Engineers

S310

#### SMARTIFICIAL CARDIAC DEVICES – CLINICIANS MEET ENGINEERS

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Modern heart failure therapy makes use of left ventricular assist devices for severe cases as well as other active implants. While these devices have come far from their beginnings with improved hemocompatibility, smaller size and less invasive implantation techniques, their advancement takes close cooperation of engineers and clinicians to face the challenges of further advancements. It is vital to ensure interdisciplinary communication and exchange to define the future of Mechanical Circulatory Support. On the engineering side, greater biocompatibility, patient-specificity and options for smart devices and telemedicine offer great potential. These developments go hand in hand with improved surgical technique and clinical expertise. This cooperation will allow us to work towards reduced mortality, less adverse clinical events and ultimately improved quality of life for the cardiac assist device patient of the future.

S315

#### COMPARATIVE ANALYSIS OF CARDIAC ENERGETICS IN ISOLATED HEARTS SUPPORTED BY PULSATILE OR ROTARY BLOOD PUMPS – IMPLICATIONS FOR CARDIAC RECOVERY?

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**Objectives:** Functional myocardial recovery during support with left ventricular assist devices (LVADs) is highly desirable but only observed in approx. 1% of all patients. Previously, the more frequently implanted pulsatile blood pumps (PBBs) showed higher recovery rates than the currently used rotary blood pumps (RBP). The aim of this study was to comparatively assess the capability of PBBs and RBPs to unload the left ventricle and restore physiological cardiac energetics in isolated hearts.

**Methods:** An RBP (Medtronic HVAD) and a heartbeat synchronized PBB (BerlinHeart Excor) were alternately connected to eight isolated porcine hearts. To modulate the mode of LVAD support, RBP rotational speed was set to different support levels and ejection delay of the PBB was phased from 0% to 90%. Pressures, flows, left ventricular volumes, and myocardial oxygen consumption were recorded and cardiac efficiency quantified by the ratio of external work (EW) over myocardial oxygen consumption.

**Results:** With increasing RBP support, lower left atrial pressures (LAP) were found to coincide with a decreased cardiac efficiency ( $r=0.91 \pm 0.12$ ) with a median [inter quartile range] slope of 0.28 [0.91] %/mmHg. In contrast, depending on the phase delay of ejection of a PBB, LAP and cardiac efficiency follow a sinusoidal course with the LAP minimum at 90% and efficiency maximum at 60%.

**Discussion:** In RBP support, aggressive ventricular unloading was found to lead to lower cardiac efficiencies. On the other side, phasing of a PBB offers the possibility to restore cardiac efficiency and simultaneously unload the ventricle. These results may justify future studies linking optimized cardiac mechanics and efficiency to functional recovery and reverse myocardial remodeling with LVAD therapy.

### Symposium: (Bio)Artificial Organs

S321

#### CELL BIOREACTOR DESIGN: BIOARTIFICIAL ORGAN DEVELOPMENT MEETS TISSUE ENGINEERING

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**Objectives:** Cells are used in bioartificial organs (BAOs) to temporarily replace the complex metabolic functions that neither failing organs, nor their artificial substitutes may provide. This makes cell bioreactors a critical technology for BAOs development. Bioreactors are used for expanding stem (SCs), precursor (PCs), or line cells to clinical mass, for promoting SCs and PCs differentiation to the needed phenotype, and for providing patients with the organ regulatory and synthetic functions needed to foster organ healing or maintain patients state until transplantation (Tx). In some cases, they are also used to regenerate or preserve tissue until Tx. The structural and functional complexity of tissues and organs, the lack of information on their pathophysiology and on disease/damage evolution, the treatment time broadly varying with patients disease and state, all make bioreactor design and operation for BAOs very challenging.

**Methods:** In spite of its complexity, cell bioreactors design for BAOs is generally based on the use of one cell type only (often line cells), scaffolds with suitable immune protective properties, and bioreactor configurations suitable for hosting clinical cell mass and intellectual property protection. Bioreactor performance has been enhanced with actuators to provide cells with mechanical, electrical and/or magnetic cues, often irrespective of tissue or organ specificity. Thus far, this approach has resulted in poor therapeutic success of the proposed BAOs.

**Results:** Cell bioreactor design for BAOs may benefit of the approaches developed over the years to bioreactor design for engineering or regenerating 3D tissue or large solid organs in vitro.

**Discussion:** Prospectively, future approaches to cell bioreactor design for BAOs should be patterned after those typical of tissue engineering and should aim to the optimization of the BAO treatment as a whole.

S322

### BIOPROCESSING ISSUES FOR MANUFACTURING OF CELL THERAPY PRODUCTS

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**Objectives:** Human immune cells have been produced using Advanced Therapy Medicinal Products (ATMP) guidelines and have been tested in clinical studies for a number of diseases, for which still no or only inadequate alternative therapies are available. Examples include several cancer entities, stroke, myocardial infarction, severe autoimmune disorders and chronic infections. Despite many in vitro or in animal studies report positive outcomes after immune cell therapy, convincing results showing the benefits of ATMP in clinical studies are rare. Part of this discrepancy in the outcome of the clinical studies in comparison to the in vitro and in animal studies may be caused by the manufacturing of immune cells for clinical trials. Cells prepared for clinical trials were mostly manufactured using archaic, scarcely controlled and incomparable production processes and stringent analytical methods for assessing proliferation and differentiation of the immune cells during the manufacturing process were not applied.

**Methods:** The production of ATMPs is first of all dependent on the donor specific biology, which significantly varies from one donor or patient, respectively, to another (biological heterogeneity). In case of immune cell therapy, the manufacturing process is key for cytotoxic function and is the basis for the biological activity of the cells after injection into the patient.

**Results:** The challenge for future clinical trials therefore is to reproducibly provide a sufficient number of biologically active cells of appropriate quality preferably in accordance with quality manufacturing guidelines (e. g. Good Manufacturing Practice, ICH etc.). Only using cells which have been produced under these rigorous quality control regimes will allow the conduction of clinically meaningful trials.

**Discussion:** The talk presents a survey and discusses consequences on future requirements for manufacturing processes of products for immune cell therapies.

S323

### EXTRACELLULAR VESICLES IN EXTRACORPOREAL THERAPIES

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**Objectives:** Extracellular vesicles (EVs) are membrane enclosed structures, which are released from all types of cells upon activation. Two examples for the role of EVs in extracorporeal therapies will be discussed in detail, (i) EVs as markers for cellular activation and (ii) EVs as platform for the binding of plasma proteins.

We assessed the impact of regional citrate anticoagulation (2.8 vs. 5.6 mM) on the cellular activation in lipoprotein apheresis, using EVs as marker for cellular activation upon blood-adsorbent interaction. Moreover, we studied the ability of a commercial adsorbent (PentraSorb) for C-reactive protein (CRP) to remove both, soluble and EV-associated CRP from septic plasma.

**Methods:** EVs were characterized in human blood or plasma via flow cytometry after calibration with fluorescent silica beads (1 µm, 0.5 µm, 0.3 µm; Kisker Biotech). Phosphatidylserine-exposing EVs were identified as lactadherin-positive events in the EV gate. Antibodies against cell specific surface markers (CD41 for platelets, CD235a for red blood cells) were used to identify the cellular origin of EVs. An anti-CRP antibody was used to identify CRP carrying EVs.

**Results:** During lipoprotein apheresis, platelet adhesion to the adsorbent polymer and EV release were associated with elevated markers for platelet activation and EV release was dependent on the citrate concentration in the extracorporeal circuit. Septic plasma contained significantly elevated levels of EVs, CRP+ EVs, as well as soluble CRP as compared to healthy donors. Incubation of plasma with the CRP adsorbent resulted in almost complete depletion of soluble CRP, and remaining EVs did not show any association with CRP, indicating detachment of CRP from the EVs.

**Discussion:** In the context of apheresis, EVs can be considered as markers for cellular activation. Moreover, our data show that EVs can serve as a platform for the binding of CRP, which has been suggested to induce conformational changes of CRP and a shift towards pro-inflammatory characteristics.

S324

### MIXED MATRIX MEMBRANE FOR REMOVAL OF UREMIC TOXINS FROM BLOOD PLASMA COMBINED TO REMOVAL OF ENDOTOXINS FROM THE DIALYSATE

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**Objectives:** For a single hemodialysis session several hundred liters of water are consumed. Water scarcity and inadequate water purification facilities worsen contamination risk especially in developing countries. Here, we investigate the application of a mixed matrix membrane (MMM), which combines filtration and adsorption, for achieving endotoxin-free dialysate combined to high removal of uremic toxins from human plasma.

**Methods:** We investigate the adsorption of lipo-polysaccharide (LPS) by a MMM in both static and dynamic conditions. Dynamic adsorption of LPS is also investigated in presence of uremic toxins in human plasma. Diffusion experiments using dialysate contaminated with bacterial culture filtrates are also performed to assess the ability of the MMM to act as a safety-barrier to avoid transfer of pyrogens to the plasma. A membrane without sorbents is used as control and the obtained results are compared with literature studies using current dialysis membranes.

**Results:** The MMM can remove approximately 10 times more endotoxins from dialysate compared to commercial dialysis membranes. No transfer of pyrogens was detected in the blood compartment, revealing safety-barrier properties of the MMM. Importantly, endotoxins from dialysate and protein-bound toxins from human plasma can be removed simultaneously without compromising AC adsorption capacity.

**Discussion:** We estimate that 0.15 m<sup>2</sup> of MMM is needed to remove the daily production of the protein-bound toxins, indoxyl sulfate and hippuric acid and to completely remove endotoxins in a wearable artificial kidney (WAK) device. Future studies would focus on the applicability of the MMM in wearable/portable artificial kidneys and in general for home-hemodialysis treatments.

**Acknowledgements:** This work is supported by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement no. 642890 (<http://thelink-project.eu/>), and it was partially supported by Health-Holland TKI project NOVAMEM (project no: LSHM16059-SGF).

**Working Group Tissue Engineering**

S331

**RESTORE HEALTH BY ADVANCED THERAPIES – A EUROPEAN LARGE-SCALE RESEARCH INITIATIVE**

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Shifting from treating symptoms to curing chronic diseases by making the transformative promise of Advanced Therapies a reality for the benefit of patients and society and by making Europe a spearhead of Advanced Therapies in Science, Clinics and Biomedical Industry, that is the vision of the large-scale research initiative RESTORE - Health by Advanced Therapies.

The increasing prevalence of chronic diseases and multi-morbidity due to demographic factors represents a high socio-economic burden for Europe. The direct health costs increased by 50% during the last decade and reached €1.526bn in 2017, a staggering 9.6 % of Europe's GDP. As current therapies rarely cure, but merely fight symptoms, never-ending treatment is required, which means diminished quality-of-life, adverse effects and soaring cost for society. There is a high need to reach sustainable improvement for patients or even to cure them of chronic diseases – in other words, to disrupt the paradigm of “treating symptoms” with “restoring health”. Advanced Therapies are the game changers that open up transforming therapeutic opportunities. For genetic diseases, immune diseases, cancer and tissue injury potential cures through Advanced Therapies exist - it is reality, not fiction. Some products are already on the market, mostly for rare diseases which means only a few thousand patients worldwide have benefitted from Advanced Therapies until now. At the advent of such a trailblazing change, obstacles and roadblocks abound. To make the disruptive promise of Advanced Therapies to cure chronic diseases a reality and to make Advanced Therapies accessible as standard-of-care for every European patient in need, RESTORE envisage to establish a sustainable pan-European ecosystem integrating transdisciplinary research, clinics, patients, and industry.

Please join us [www.restore-horizon.eu](http://www.restore-horizon.eu) for more details. RESTORE is funded by the EU for the preparatory phase of the large-scale research initiative (h2020, No 820292).

S332

**COMBINATION OF NANOSTRUCTURED SURFACES MADE BY LASER INTERFERENCE LITHOGRAPHY WITH LAYER-BY-LAYER TECHNIQUE TO GUIDE CELL STEM CELL DIFFERENTIATION**

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**Objectives:** Laser interference lithography (LIL) and the layer-by-layer (LbL) technique are combined here for the first time to design a system with variable nanotopographies and surface viscoelasticity to regulate cell behavior.

**Methods:** LIL is used to generate hexagonally arranged nanostructures of gold with different periodicity. In contrast, LBL is used to assemble a multilayer system of poly-L-lysine (PLL) and hyaluronic acid (HA) on top of the nanostructures. Moreover, the viscoelastic properties of that system are controlled by chemical cross-linking.

**Results:** We show that the topography designed with LIL is still present after multilayer deposition and that the formation of the multilayer system renders the surfaces hydrophilic, which is opposite to the hydrophobic nature of pristine nanostructures. The heterogenic system is applied to study the effect on adhesion and differentiation of human adipose-derived stem cells (hADSC). We show that hADSC spreading is increasing with cross-linking degree on flat multilayers, while it is decreasing on nanostructures modified with multilayers. In addition, early effects on signal transduction processes are seen. Finally, hADSC differentiation into chondrogenic and osteogenic lineages is superior to adipogenic lineages on nanostructures modified with multilayers.

**Discussion:** Hence, the presented system offers great potential to guide stem cell differentiation on surfaces of implants and tissue engineering scaffolds.

**Acknowledgements:** This work was supported by Deutsche Forschungsgemeinschaft grant Gr 1290/10-1 and Bundesland Sachsen-Anhalt in the frame of Leistungszentrum Chemie- und Biosystemtechnik (project N°. H01127247).

S333

**FUNCTIONAL NANOFIBROUS SCAFFOLDS COMBINED WITH STEM CELLS FOR ADVANCED BIOMEDICAL DEVICES AND THERAPIES**

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Among the various possible embodiments of Advanced Therapies and in particular of Tissue Engineering the use of temporary scaffolds to regenerate tissue defects is one of the key issues. The scaffolds should be specifically designed to create environments that promote tissue development and not merely to support the maintenance of communities of cells. To achieve that goal, highly functional scaffolds may combine specific morphologies and surface chemistry with the local release of bioactive agents.

Many biomaterials have been proposed to produce scaffolds aiming the regeneration of a wealth of human tissues. We have a particular interest in developing systems based in biodegradable polymers. Those demanding applications require a combination of mechanical properties, processability, cell-friendly surfaces and tunable biodegradability that need to be tailored for the specific application envisioned. Those biomaterials are usually processed by different routes into devices with wide range of morphologies such as biodegradable fibers and meshes, films or particles and adaptable to different biomedical applications.

In our approach, we combine the temporary scaffolds populated with therapeutically relevant communities of cells to generate a hybrid implant. For that we have explored different sources of adult and also embryonic stem cells. We are exploring the use of adult MSCs, namely obtained from the bone marrow for the development autologous-based therapies. We also develop strategies based in extra-embryonic tissues, such as the perivascular region of the umbilical cord (Wharton's Jelly).

We are currently involved in a European consortium aiming at developing films with antimicrobial properties to be used in hospitals to cover surfaces that are prone to facilitate the transmission of infections. Those films use surface topographies and natural-derived oils to ensure the needed antibacterial properties to the films.

This talk will review our latest developments of functionalized biomaterials and scaffolds in combination with stem cells for advanced biomedical devices and therapies.

**Acknowledgements:** The author acknowledges the Portuguese Foundation for Science and Technology (FCT) for the FCT Doctoral Program on Advanced Therapies for Health (PATH) (FSE/POCH/PD/169/2013) and the financial support of the projects SPARTAN (PTDC/CTM-BIO/4388/2014), FRONThERA (NORTE-01-0145-FEDER-0000232) and FLEXPOL (H2020-NMBP-PILOTS-2016-721062).

S334

### STRATEGIES TOWARDS EFFICIENT CRYOPRESERVATION OF CELL-FREE AND CELL-SEEDS SCAFFOLDS

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**Objectives:** Cryopreservation of 'ready-to-use' tissue-engineered constructs (TECs) is a promising strategy which may facilitate their future clinical application. This is very challenging and ambitious task and therefore recent efforts have been focused on developing new cryopreservation

strategies for long-term storage of TECs. This work covers some practical considerations for cryopreservation of cell-free and cell-seeded scaffolds vastly differing by structure and composition.

**Methods:** The first test system includes 3D porous collagen-hydroxyapatite (HAP) scaffolds prepared by freeze-drying and coaxial alginate microspheres prepared by electrospraying. Samples were frozen at 1 K/min either in a bulk DMSO solution (with and without sucrose) or after removal of residual solution. After thawing, we evaluated compression (collagen-HAP scaffolds) and rheological properties (coaxial alginate microspheres) of cell-free systems. Viability of mesenchymal stromal cells (MSCs) within both types of scaffolds was evaluated 24-h post-thaw using live-dead assay. The second test system comprises flat fiber mats (produced from polycaprolactone/poly(lactic acid) using electrospinning) seeded with CHO cells. This system intends to develop plate electrodes for electroporation of attached cells with non-permeable cryoprotective agents (CPAs) such as sugars for future cryopreservation applications.

**Results:** All scaffolds were cytocompatible with corresponding cell types. Freezing after removal of residual solution was superior to conventional freezing. Addition of sucrose increased cell viability (both scaffold types) and improved viscoelastic properties of coaxial microspheres. Constructed plate electrodes provided good compromise between high cell permeabilisation and viability after electroporation with sucrose at 1.7 kV/cm electric field.

**Discussion:** The findings suggest that it is feasible to cryopreserve cell-free and cell-seeded scaffolds using DMSO and sucrose. As a step further, there are high expectations associated with using electroporation as a mean for intracellular delivery of non-toxic CPAs towards DMSO-free cryopreservation of TECs.

**Acknowledgements:** IP@Leibniz Program of Leibniz University Hannover (DAAD project code 57156199) as well as Ways to Research Program of the Leibniz University Hannover (60442522). We also acknowledge support of our students Diaa Khayat and Tobias Pfister.

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