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Meeting Report

2nd Centro3R Annual Meeting: 3Rs in Italian Universities

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A year after its creation, promoted by the Universities of Genoa and of Pisa (UniGE and UniPI), the Interuniversity Center (Centro3R, <https://www.centro3r.it/en>) boasts some 300 members from Turin and Milan Polytechnic (PolyTO and PolyMI), the Milan-Bicocca (MIBi) and Pavia University (UniPV). On June 20-21, 2019, the 2nd Annual meeting “3Rs in Italian Universities” was organized by the Genoa Operative Unit, headed by **Anna Maria Bassi**, with support from other Centro3R members. The meeting of 110 participants was hosted with the generous support of the Genoa University Rector, Prof. **Paolo Comanducci**. Several companies involved in *in vitro* advanced technologies and cellular models also supported the meeting: IvTech, Jove, MatTek, UPM-Biofore, Prodotti Gianni, Euroclone, Tebu-bio and Voden. The meeting included talks by representatives from European and national institutions, by members of each Centro3R Operative Unit, as well as by other research institutions and companies engaged in the field of innovative technologies and alternative methods.

To promote sustainability of the 3R principles in 21st century science, the Centro3R Scientific Committee supported the participation of young scientists to present their research activities within the Centro3R Operative Units, which encouraged the submission of abstracts from 30 young and upcoming researchers. Taking into account 3R relevance and scientific value, the Scientific Committee awarded reimbursement for the conference accommodation for the six best abstracts to Francesco Biagini (UniPI), Marco Campisi (PolyTO), Ayesha Idrees (PolyTO), Daniela P. Pacheco (PolyMI), Lorenzo Sardelli (PolyMI) and Sara Tirendi (UniGE). Bonuses offered by contributing sponsors were awarded for the six best poster presentations to Ludovica Cacopardo (UniPI), Alice Zoso (PolyTO), Mara Terzini (PolyTO), Stefano Gabetti (PolyTO), Monica Oldani (MIBi) and Roberta Nossa (UniPI). All winners also received a certificate.

The meeting was opened by Prof. **Arti Ahluwalia**, director of the Centro3R, and Anna Maria Bassi following the inaugural speeches of the Rector of the University of Genoa and the Vice Mayor. **Susanna Louhimies**, DG Environment Brussels, gave an informative keynote speech on Directive 2010/63/EU, focusing on progress, challenges and future direction. The importance of implementing existing 3Rs approaches through a more careful project evaluation, through training of project evaluators, and by improving efficiency and consistency with the support of national committees was emphasized as was the role of 3Rs centers in creating a strong strategic network. On the second day, the key

note speaker **Chantra Eskes** presented the work of the Swiss 3R Competence Center (3RCC), which comprises 11 universities and several higher education institutions, in research, education and communication implementation as well as the center’s 3Rs research funding program.

The topic *Involvement of national and international institutions in 3R implementation* was covered by **Ugo Santucci** (Italian Ministry of Health), **Rodolfo Lorenzini** (Superior Institute of Health) and **Giuseppe Fioroni** (Italian Ministry of Education, University and Research), who confirmed their full institutional backing of the 3Rs. **Adelaide Dura** (Joint Research Centre), called for better education, communication and outreach within the 3Rs to enhance knowledge-sharing and promote its uptake. In particular, she emphasized the JRC’s work on compiling an extensive overview of the 3Rs education and training landscape by mapping available courses and resources worldwide and by launching a project to investigate the feasibility of including the 3Rs in educational curricula under a European Parliament Pilot Project, which aims to promote alternatives to animal testing.

For the topic *Scientific, ethical and legal issues of reduction and refinement*, **Angelo Gazzano** (UniPI) reviewed the refinement of laboratory animal welfare by identifying the more respectful methods of handling and housing of animals. **Michele Panzera** (University of Messina) spoke about neurophysiological models of animal sentience. He explained that basic functional homologies can be mapped to the brain regions directly responsible for pain sensation stimuli and their representation in the sentience limbic circuits. **Franco Manti**’s lecture (UniGE) was on moral arguments for an ethical experimentation. He presented the concept that effective development of the 3Rs requires epistemological awareness and ethical competence for which responsibility must be assumed by researchers and members of animal welfare bodies.

The topic *Companies supporting 3R principles* was covered by four young Italian researchers who are working in companies that are involved in advanced 3R models and technologies. **Salvatore Simmini** (Stemcells Inc. Corp.) gave a lecture on organoids, highlighting their use in medical research and their potential to reduce the number of animals required for experimentation. **Nikolas Gaio** (BIOND Solutions B.V. Netherlands) explained the features of organ-on-chip platforms, which combine human cells with microfluidic chips to precisely control cell culture conditions, and how they are applied in oncology, neurodegenerative and cardiotoxicity studies. **Francesco Nevelli** (Merck



Group, Italy) presented data on a replacement project aiming to mimic the physiological action mechanisms of gonadotropin and growth hormone. **Alberto Manganaro** (Kode Chemoinformatics S.r.l., Italy) outlined the need and demand for chemoinformatic approaches for quick, reliable and inexpensive screening and assessment of chemicals, in particular for regulatory activities, such as several software tools for QSAR aimed at ecotoxicological assessments.

The topic *In silico models: Toxicology & efficacy of drugs and chemicals* was opened by **Marco Agostino Deriu** (PolyTO), who gave a talk on computational molecular modelling in rational drug design and discovery, explaining that the structural features of protein aggregation at a molecular level may provide novel opportunities for overarching therapeutic approaches, such as blocking common aggregation-induced cellular toxicity pathways. **Gaetano Valenza** (UniPI) gave a brief explanation and some statistical insights on why higher statistical significance does not automatically imply better predictivity and illustrated how to complement data analysis using machine learning. He pointed out that these techniques can be exploited to derive insights on pathophysiology. **Luigi Preziosi** (PolyTO) described how mathematical tools can support cancer research, e.g., by classifying and understanding omics data, foreseeing the evolution of a biomedical system, explaining how pathologies develop or optimizing therapies.

The topic *Application of reduction and refinement principles in animal testing* was addressed by **Raffaella Colombo** (UniPV), who reported the features of a new *in vitro* gastrointestinal platform to evaluate the effects of exogenous molecules using a dynamic millifluidic system. **Manuela Marcoli** (UniGE) introduced the pilot Centro 3R Organ and Tissue Sharing Project, which involves the University of Genova and of Pisa and their animal-welfare bodies, and pointed out the importance of sharing organs and tissues from animals humanely sacrificed for (scientific or research) projects approved by the Ministry of Health, allowing the researcher to use valuable biological resources in the best way. The project aims to contribute to reducing animal use for experimental purposes. **Michele Conti** (UniPV) described how the combination of *in vitro* tests performed using *ex vivo* tissue, integrated, validated patient-specific numerical simulations, and mock arteries manufactured by 3D printing can offer important insights on the biomechanical impact of endograft design on post-operative aortic mechanical response. **Giovanni Nicoletti** (UniPV) followed by reviewing the regenerative effects of platelet rich plasma on human skin wound repair and the regeneration process. **Gianluca Ciardelli** (PolyTO) highlighted the relevance of the transferability of tissue engineering technologies to the design of tissue models, emphasizing that 3D tissue-engineered models are promising tools in the screening and evaluation of drugs and therapies, as well as in the investigation of the molecular mechanisms involved in disease onset and progression.

For the topic *Disease models using human cells, tissues and organs*, **Barbara Parodi** (IRCSS San Martino Hospital Genoa) focused her talk on BBMRI-ERIC, a European research infra-

structure for biobanking that brings together researchers, biobankers, the industry and patients. Twenty countries are currently part of BBMRI-ERIC, and the Italian node includes 90 biobanks/biological resource centers/collections. **Simone Lapi** (Pisa Biobank and Tuscany Regional Biorepository) gave an overview on the activities of the Regional Biological Archive, a biobank that stores serum, plasma and non-viable cells of multi-organ/tissue donors as well as solid organ transplant recipients from the Tuscany Region, providing the possibility to review and re-evaluate the donors and recipients, even after some years. **Gianfranco Beniamino Fiore** (PolyMI) outlined the features of advanced culture systems for *ex vivo* vascular tissue conditioning and illustrated how bioengineering approaches can speed up the development of life-saving treatments. He pointed out that the use of human samples, particularly excess surgically-derived samples, is a valuable approach that is worth the high setting-up and management costs.

The topic *Application of replacement* was opened by **Maria Grazia Cascone** (UniPI), who emphasized the relevance of tissue-engineered constructs as animal replacements and highlighted how biomimetics and microfluidics can be used to build *in vitro* 3D systems. **Sara Mantero** (PolyMI) reported on the use of bioreactors for the *in vitro* development of engineered substitutes, and the uses of *in vitro* 3D models that can be considered as a bridge between 2D models and *in vivo* models. **Paola Petrini** (PolyMI) described research focused on hydrogel-based platforms used to mimic *in vivo* drug diffusion. An airway mucus model was proposed as a high-throughput *in vitro* drug screening tool, which can also be used to study the effects of bacteria. **Susi Burgalassi** (UniPI) presented on the evaluation of reconstituted epithelial corneal tissues for drug delivery by comparing a lab-made artificial corneal epithelium with a commercially-made human corneal epithelial model for the evaluation of ocular permeation of drugs and drug formulations.

For the topic *Commitment of Centro 3R in university training*, **Arti Ahluwalia** (UniPI) underlined the importance of including the 3R principles in the core curricula and the hurdles that must be overcome to achieve this. **Anna Maria Bassi** (UniGE) gave insight into her experience in teaching and training in substitutive approaches to animal testing, including training courses, lectures within teaching programs, work experience opportunities for graduate or graduating students in biology, medicine and surgery as well as PhD courses on human *in vitro* model disease projects. These efforts have been greatly enhanced by Centro3R activities by increasing the awareness of alternative approaches. **Valeria Chiono** (PolyTO) introduced a new 30-hour course on the 3Rs, "New advances in alternative preclinical trials" as an optional course for master students in biomedical engineering. Finally, **Andrea Aliverti** (PolyMI) introduced the course "Ethics in Research" for PhD training, which teaches how to identify ethical issues, how to interpret, assess, and apply various research rules, and how to make decisions and to act ethically in various situations.

The meeting was concluded with a roundtable on the role of 3R centers, consolidating the scientific network on 3R teaching

and research. The multi-disciplinary nature of the speeches was highly appreciated and paved the way for synergic collaborations among the participants. The conference attracted the interest of numerous researchers as well as the media, who greatly contributed to the dissemination of Centro3R's mission and promoted it as a major reference point for research and teaching resources in Italian academia.

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Meeting Report

Swiss-Chinese Cooperation for Organs-on-a-Chip and Stem Cell Research

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Sino-Swiss Workshop for Tissue Engineering

Organ-on-a-chip (OOC) systems, or microphysiological systems (MPS), are a new biomedical research field that aims to recapitulate organ-level tissue structures and organ functions for drug evaluation and disease modelling. The research is gaining importance in the context of further advancements in the development and implementation of organotypic models for drug development. The Annual Meeting of the TEDD network (Tissue Engineering for Drug Development and Substance Testing) took place in October 2019 in Wädenswil, Switzerland. We hosted researchers from Southeast University (SEU), China, National Institutes of Health (NIH), USA, Chinese University of Hong Kong (CUHK), and Swiss academics and industrial research groups active in the field of tissue engineering to build a strategic research and implementation alliance. Speakers presented the latest findings on OOC, 3D cell culture, stem cells and bioprinting. On the podium, we discussed potential funding sources, programs and collaborations with European and international networks. The aim was to accelerate the translation and validation process of current tissue engineering technologies. Additionally, representatives of swissnex and Swiss National Science Foundation (SNSF) talked about how governmental institutions support re-

search collaborations between China and Switzerland. Preceding the meeting, the international scientists visited leading research groups and companies in Switzerland.

The Chinese delegation included Prof. **Zhongze Gu** and Prof. **Ningping Huang** from the School of Biological Science and Medical Engineering, Southeast University, Nanjing and Dr **Zaozao Chen**, a technical director from the Institute of Biomedical Devices (IBMD), Suzhou. Prof. Zhongze Gu's group has developed multiple OOC and MPS systems, including blood vessels, heart, kidney, and liver tissue. The miniature organs made with advanced microfabrication, 3D bioprinting, microfluidics, and tissue engineering techniques form tissue-specific structures and maintain desirable organ functions for more than four weeks. Current research is aimed at the development of an advanced 3D read-out system that can characterize OOC tissues and analyze their morphology and functional features automatically in a quantitative manner. For example, this system could offer a package solution to analyze a tumor spheroid's/organoid's viability and invasiveness, together with the prediction of drug classification and mechanism using deep-learning-based artificial intelligence (AI)-algorithms. Thus, this system could be useful for oncology drug screening and evalu-