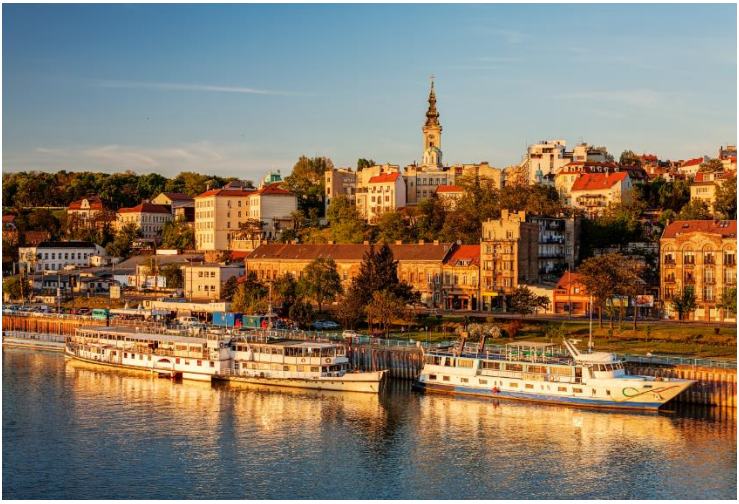
“Cells Communicate!”

Book of Abstracts of the Second CellFit Workshop 2019



Belgrade, Serbia 13 – 14 March, 2019

Editors:

Tiziana Brevini, Alireza Fazeli, Bart Gadella, Sergio Ledda, Mirjana Mihailović, Melita Vidaković, Aleksandra Jauković, Tamara Kukolj

We would like to thank the following organizations for their support:

European Cooperation in Science and Technology

COST is supported by the EU Framework Programme Horizon 2020

University of Life Sciences

Publishers: Institute for Biological Research „Siniša Stanković", University of Belgrade and the COST Action CA16119, Brussels
Book Title: Cells communicate!
Year of Publication: 2019
Print: Copy Planet, Belgrade, Serbia
Circulation:70
ISBN 978-86-80335-08-7 (print)

Version: V5

Table of Contents

About the European Co-operation in Science and Technology

The European Cooperation in Science and Technology (COST) is the oldest and widest European intergovernmental network for cooperation in research. Established by the Ministerial Conference in November 1971, COST is presently used by more than 30,000 scientists of 35 European countries to cooperate in common research projects supported by national funds. The financial support for cooperation networks (COST Actions) provided by COST is about 1.5% (30 million EUR per year) of the total value of the projects (2,000 million EUR per year). The main characteristics of COST are:

- bottom up approach (the initiative of launching a COST Action comes from the European scientists themselves),
- à la carte participation (only countries interested in the Action participate),
- equality of access (participation is also open to the scientific communities of countries which do not belong to the European Union) and
- flexible structure (easy implementation and management of the research initiatives).

As a precursor of advanced multidisciplinary research, COST has a very important role in shaping the European Research Area (ERA). It anticipates and complements the activities of the current Framework Programme for Research and Innovation (Horizon 2020). COST activities create a bridge between the scientific communities of countries and increases the mobility of researchers across Europe in many key scientific domains.

Welcome from the chairman

Welcome to the second Workshop of our COST Action CA16119, that will take place in the capital of Serbia. Known as “the city that never sleeps”, Belgrade is one of the oldest cities in Europe and the only one built on the confluence of two rivers – the Danube and the Sava.

During these days, we will enjoy great scientific sessions, with distinguished speakers from many different COST countries. We will discuss how cells communicate. We will analyze the effect played by the microenvironment and better understand how cells react to biomechanical cues.

I do hope this second meeting will encourage collaborations and will further implement synergies and networking within the Action participants, especially the early career ones.

I would like to express my deepest gratitude to Melita Vidaković and all the others of the local organizing committee for the fantastic work they have done in planning this event. Also, many thanks to our WG2 Leaders, Bart Gadella and Sergio Ledda, for their input in

composing the program and to Shaghayegh Basatvat for managing the technical aspects.

Last, but not least, many thanks to all of you, for your enthusiastic help, trust and support.

See you in Belgrade!

Добродошли у Београд!

Prof Tiziana A.L. Brevini
Chair of CellFit
March 2019

Welcome address from the local organiser

Dear CellFit members,

Welcome to Belgrade, the cultural and scientific center of Serbia. It will delight you with the warm hospitality and unique atmosphere reflecting the fusion of different cultures.

Belgrade has been a university town for a long time within which various scientific institutes found fruitful ground. As the members of the University of Belgrade, Institute for Biological Research and Institute for Medical Research have been recognized as leading national scientific institutes in the field of biological and medical sciences committed to the complex state of the art research and multidisciplinary approach.

We are truly delighted about the given opportunity to jointly host the second workshop of the COST Action CA16119 CellFit focused on different aspects of dynamic cell to cell interactions. This meeting will fortify our knowledge on how intercellular communication along with various biochemical and mechanical cues influence cells

behavior which might bring us step closer to engineer 3D physiological microenvironment *in vitro* and overcome the drawbacks associated with 2D models. Moreover, we believe that this event will strengthen the collaboration and encourage the exchange of ideas between the scientists through insightful discussions.

We look forward to have an inspiring meeting in Belgrade.

Melita Vidaković, Mirjana Mihailović, Aleksandra Jauković, Diana Bugarski

Programme

Day 1, March 13th, 2019: Arrival to Belgrade and check in at Hotel Moskva. Welcome dinner at FishZelenis at 19:30h (http://www.fishizelenis-bg.com/).

Day 2, March 14th, 2019: Workshop *"Cells Communicate!"*

8:00-9:00
The registration desk is open at the hotel Moskva Conference room

09:00-09:15
Welcome address and introduction (Prof. Tiziana Brevini and local organizers)

09:15-09:45
Prof. Vladislav Volarević, Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Serbia
„Mesenchymal stem cell-based therapy: importance of communication with T regulatory cells"

09:45-10:15
Prof. Giovanna Brusatin, University of Padova, Italy
„Biomaterials and engineered microenvironments to control YAP/TAZ-dependent cell behaviour"

10:15-11:00 ***Coffee break and Poster session***

11:00-12:00

Six selected oral presentation (10 min) from submitted Abstracts (Abstract number: **O1 to O6**)

1. Khnykin Denis: 3D organotypic skin models: mimicking the largest organ in the body in vitro

2. Radostina Alexandrova: Application of 2D and 3D cell cultures in cytotoxicity assessment of new metal complexes with kojic acid

3. Joana S. Rodrigues: Culturing hepatocytes-like cells in microfluidic devices: a new tool for drug discovery, disease modelling and toxicology

4. Milda Alksne: Macro-structured polylactic acid scaffolds for osteogenic regeneration

5. Nicolás M. Ortega: Bovine preimplantation embryos at the single cell level: An accurate model for human reproductive approaches?

6. Martina Štampar: Application of the comet assay on 21-days old spheroids developed from HepG2/C3A cells

12:00-13:00 ***Lunch***

13:00-13:30

Prof. dr. Peter Ertl, *Vienna University of Technology*, Austria

"Next Generation Organ-on-a-Chip Systems Incorporating Biomechanical Cues"

13:30-14:00

Dr. Marta Munoz, SERIDA, Spain

"Cell to cell and social media communication more alike than you would think"

14:00-15:00

Six selected oral presentation (10 min) from submitted Abstracts (Abstract number: **O7 to O12**)

1. András Dinnyés: Modelling the neuropathology of mucopolysaccharidosis type II with diseasespecific
human induced pluripotent stem cells

2. Jolanda VAN HENGEL: Cell communication in cardiac muscle cells

3. Agnieszka Waclawik: Prostaglandin F2α stimulate endothelial cell network formation and the
gene expression of angiogenic factors in the porcine endometrium

4. Sharon Arcuri: 3D cell rearrangement promotes DNA hypomethylation and maintains high
plasticity of epigenetically erased adult somatic cells

5. Furtos Gabriel: Mechanical and biological activity on properties of new bone cement

6. Sharon Schlesinger: Mesenchymal stem cells modulate the inflammatory response of mammary
epithelial cells

15:00-16:00 ***Coffee break and Poster session***

16:00-16:15 Any open questions related to CA16119

16:15-16:30 Wrap up, discussion

19:00-22:00

Gala Dinner at boat "SIRENA". Cruise at the Sava and the Danube river with nice orchestra from Vojvodina.

Day 3, March 15th, 2019: Participant's departure

Optional tourist programme:

10:30 h - **BELGRADE SIGHTSEEING TOUR-BELGRADE WALKS** (http://www.tob.rs/belgrade-sightseeing-tours/belgrade-walks/belgrade-walking-tours). Duration: 2,5 h.

Abstracts of the CellFit Workshop

- Invited speakers – Abstracts: I1-I4
- Selected abstracts for the oral presentation: O1-O12
- Selected abstracts for the poster presentation: P1-P29

I1

Mesenchymal stem cell-based therapy: importance of communication with T regulatory cells

Vladislav Volarevic

Department of Microbiology and Immunology, Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Serbia

Abstract.
Background: Mesenchymal stem cells (MSC) are self-renewable cells with immunomodulatory characteristics. Accordingly, MSC-based therapy showed promising results in experimental models of acute and chronic inflammatory diseases. MSC inhibit detrimental immune response either directly or through the interaction with other immunosuppressive cells, particularly T regulatory cells (Tregs) which, in juxtacrine and paracrine manner, suppress inflammation and promote tissue repair and regeneration.
Materials and method: We used murine models of fulminant hepatitis, liver fibrosis and acute kidney injury (AKI) to elucidate molecular mechanisms which are responsible for MSC:Tregs cross-talk in the inflamed liver and kidney. Biochemical, histological analysis, enzyme-linked immunosorbent assay (ELISA), immunohistochemistry, real-time PCR, flow cytometry and intracellular staining of liver and renal -infiltrating immune cells, selective depletion of Tregs as well as adoptive trasnsfer of MSC-primed Tregs were used to delineate the importance of MSC:Tregs

communication for MSC-based beneficial effects in the therapy of liver and renal inflammatory diseases.
Results: MSC-dependent attenuation of liver injury, fibrosis and AKI in mice was accompanied with an increased presence of interleukin (IL) 10-producing CD4+ CD25+ forkhead box P3+ Tregs and with attenuated the capacity of liver and renal -infiltrated immune cells (natural killer T (NKT), dendritic cells (DCs), CD4+T cells) to produce inflammatory cytokines. MSC promote expansion of Tregs in paracrine, inducible nitric oxide synthase- and indoleamine 2,3-dioxygenase-dependent manner. MSC-priming of Tregs significantly enhanced their immunosuppressive potential. Adoptive transfer of MSC-primed Tregs showed better efficacy compared to MSC-unprimed Tregs. Additionally, selective depletion of Tregs remarkably abrogated MSC-dependent attenuation of inflammatory immune response in the liver and the kidney, suggesting the importance of Tregs for MSC-based hepatoprotective and nephroprotective effects.
Conclusion: Cross-talk between MSC and Tregs is crucially important for the attenuation of liver and kidney inflammatory diseases.
Keywords: mesenchymal stem cells; T regulatory cells, inflammation, liver, kidney

I2

Biomaterials and engineered microenvironments to control YAP/TAZ-dependent cell behaviour

Giovanna Brusatin, PhD

University of Padova, ITALY

Abstract. Development of cell culture systems are indispensable for advancing in basic biology and clinical translations. Breakthroughs have been discovered using 2D with defined and controlled physical properties such as stiffness and geometry, evidencing that signals that cells receive from the physicality of their microenvironment are absolutely essential for their survival and to direct their fate. These results go far beyond the limit of the classically preferred culture model, 2D cell monolayers cultured on adhesive rigid and flat plastic petri dish substrates. However, cells grown in vivo within a complex 3D soft microenvironment and 3D cultures have been more recently introduced for in vitro studies, showing structurally and functionally different behavior of embedded cell aggregates or organoids.

At the meeting, I will introduce examples of engineered biomaterials and microenvironments to control cell-behavior using mechano-transcriptional regulators, YAP and TAZ, as molecular beacon of the cell response. The use of chemically defined biomaterials for the preservation of pancreatic progenitor traits ex-vivo, 2D hydrogels with controlled rigidity and micropatterned substrates to control cell behavior, will be presented.

The engineering of in vitro 2D and 3D culture microenvironments still requires efforts to develop reproducible and chemically/physically defined biomaterials, in particular hydrogels, and to use microfabrication techniques to generate controlled shapes and microenvironment, which more closely mimics key aspects of the natural environment of cells. New opportunities in these directions will be discussed.

G. Brusatin, T. Panciera, A. Gandin, A. Citron, S. Piccolo, Biomaterials and engineered microenvironments to control YAP/TAZ-dependent cell behaviour. Nat Mater, 17, 1063-1075 (2018).

L. Chang, L. Azzolin, D. Di Biagio, F. Zanconato, G. Battilana, R. Lucon Xiccato, M. Aragona, S. Giulitti, T. Panciera, A. Gandin, G. Sigismondo, J. Krijgsveld, M. Fassan, G. Brusatin, M. Cordenonsi, S. The SWI/SNF complex is a mechanoregulated inhibitor of YAP and TAZ. Nature 563, 265-269 (2018).

I3

Next Generation Organ-on-a-Chip Systems Incorporating Biomechanical Cues

Peter Ertl

Vienna University of Technology (TUW), Faculty of Technical Chemistry, Getreidemarkt 9, A-1060 Vienna Austria

Abstract. Microfluidics is both the technology to fabricate microdevices and the science to study the behavior of fluids, (bio)chemical reactions and biological responses at the microscale. In light of the benefits of microfluidics, my research group at TUW is developing lab-on-a-chip systems containing integrated fluid handling, degassing, mechanical actuators and biosensing systems to non-invasively monitor dynamic cell population responses. We have successfully integrated different electro-analytical, magnetic and optical detection methods in microfluidic devices to detect cell-to-cell and cell-to-matrix interactions. In course of the presentation advantages and disadvantages of various cell-based lab-on-a-chip systems capable of providing biomechanical forces will be discussed. Additionally a number of applications including cell migration assays, allergic responsens, shear force dependent nanoparticle uptake as well as the formation of pre-vascular networks will be presented.

I4

Cell to cell and social media communication more alike than you would think

Muñoz M[1], Przyborski S[2,3], Gómez E[1].

[1] *Genética y Reproducción Animal, SERIDA, Sp*
[2] *Department of Bioscience, Durham University, UK*
[3] *Reprocell Europe, Glasgow, UK*

Abstract. Social media has changed the way we communicate. In January 2019, there were 3.397 billion users worldwide and the 3 leading social media sites had 5.130.000 monthly users. It is not infrequent that young people use more than one social media platforms; for instance, 18-29 year old people uses on average 4 platforms.

In recent years the discovery of new mediators in intercellular communication -as exosomes- is also changing our knowledge about how cells interact with their environment and other cells. Interestingly, cells, as media users, proceed through different ways to communicate, which depends on their "target audience".

Studies employing three-dimensional (3D) cell culture systems are emphasizing that cells in 3D culture behave morphologically and physiologically in different ways than in 2D culture. It is also likely that the additional dimensionality of 3D cultures and the 3D culture type (anchorage dependent, anchorage independent …) might change the mechanisms that cells use to communicate.

3D cell culture systems allow developing new strategies for drug discovery, tissue engineering, cancer research and in many others fields. Thus, one will need to choose the most

appropriate system for their specific application. However, each 3D culture system has their own strengths and limitations. We will discuss the implications of using different synthetic scaffolds to develop 3D endometrial cell culture model.

O1

3D organotypic skin models: mimicking the largest organ in the body in vitro

Khnykin Denis

Oslo University Hospital

Abstract. Skin is a biggest organ in the human body that plays an important role as a physical barrier and has many other complex functions. Most in vitro studies in experimental skin biology have been done in 2-dimensional (2D) monocultures, while it became evident that cells behave differently when they are grown within a 3D extra-cellular matrix and also interact with other cells. Three-dimensional skin models offer the potential to study normal physiological skin features and mechanisms of various skin diseases, along with testing of compounds in vitro. In my presentation I will describe existing 3D skin models in terms of their basic characteristics and testing applications to study skin irritation, phototoxicity, dermal absorption, skin sensitization as well as for tissue engineering and drug testing.

O2

Application of 2D and 3D cell cultures in cytotoxicity assessment of new metal complexes with kojic acid

Milena Glavcheva[1], Zdravka Petrova[1], Rossen Spasov[1,2], Desislav Dinev[1], Boyka Andonova-Lilova[1], Tanya Zhivkova[1], Lora Dyakova[3], Abedulkadir Abudalleh[1], Gabriela Marinescu[4], Daniela-Cristina Culita[4], Luminita Patron[4], Radostina Alexandrova[1]

[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 25, 1113 Sofia, Bulgaria
[2]Faculty of Medicine, Sofia University "St. Kliment Ohridski", 1 Koziak Str., 1407 Sofia, Bulgaria
[3]Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 23, 1113 Sofia, Bulgaria
[4]Institute of Physical Chemistry "Ilie Murgulescu", Romanian Academy, Splaiul Independentei 202, 060021 Bucharest, Romania

Abstract. Monolayer (2D) cell cultures are widely used in routine laboratory practice and play an important role in our understanding of cancer biology. They also have been used extensively in the discovery and characterization of new antineoplastic drugs. This is not surprising because 2D cell cultures possess many advantages - they are well established, less expensive and easier to maintain, there is a lot of literature available in the field and monolayer cultures allow easier environmental control. On the other hand three-

dimensional (3D) cell cultures are suggested to be more physiologically relevant and predictive cell model systems.
The aim of our study was to evaluate the influence of newly synthesized complexes of Zn(II), Co(II) and Ni(II) with kojic acid on 2D and 3D growth of human HeLa cervical carcinoma cells. The investigations were performed in: short-term experiments (24-72 h, with monolayer cell cultures) carried out by thiazolyl blue tetrazolium bromide (MTT) test, neutral red uptake assay (NR), crystal violet staining (CV), double staining with acridine orange and propidium iodide, AnnexinV/FITC method; ii) long-term experiments (14 days, with 3D cancer cell colonies) performed by 3D-colony forming method. The results obtained revealed that the compounds examined decreased viability and proliferation of the treated cells in a time- and concentration-dependent manner in various levels. Co(II) complex with kojic acid (CoKoj) was found to be the most promising cytotoxic agent whereas NiKoj exhibited the lowest cytotoxicity. Cytopathological changes and apoptosis were observed in HeLa cells cultivated in the presence of CoKoj. The CC50 of this compound determined after 72 h of treatment by MTT test, NR assay and CV staining was estimated to be 15.3 μM, 23.7 μM and 11.1 μM, respectively. Applied at concentrations ≥ 170 μM CoKoj completely inhibited 3D colony forming ability of HeLa cells in a semisolid medium.

O3

Culturing hepatocytes-like cells in microfluidic devices: a new tool for drug discovery, disease modelling and toxicology

Joana S. Rodrigues[1], Madalena Cipriano[1], Jorge C. Correia[2], Sérgio P. Camões[1], Matilde Castro[1], Jorge L. Ruas[2], Joana P. Miranda[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisbon, Portugal;
[2]Department of Physiology and Pharmacology, Molecular & Cellular Exercise, Physiology Unit, Karolinska Institutet, Stockholm, Sweden

Abstract. Metabolic syndrome is an interplay between liver, skeletal muscle, and adipose tissue. Therefore, it is important to uncover tissue communication within this disease, including the role of hepatocytes. Indeed, microfluidic devices (MDs) provide the possibility of studying the communication between different cell types and of better mimicking human physiology. However, moving traditional in vitro 2D cultures to MDs is challenging. As such, this work focused on the adaptation of human umbilical cord matrix MSCs-derived hepatocyte-like cells (HLCs) to double channel PDMS-based MDs, evaluating hepatic-specific function and energy metabolism. 2D static cultures of HLCs and primary human hepatocytes were used as controls. HLCs were obtained in 2D through a 3-step differentiation protocol lasting 21 days. Cells were seeded at day 17 (D17) in MDs for hepatic maturation. Both coating and cell inoculum were

optimized and HLCs' albumin and urea production were evaluated up to D34 along with expression of genes involved in glycolysis, gluconeogenesis, fatty acid and bile acid metabolism and mitochondrial function in response to 80 nM insulin, 100 mM of glucagon and fasting. Herein, HLCs were successfully adapted to MDs by inoculating 7.5 x 104 cells/channel using 0.2 mg/mL of type I collagen as coating. HLCs presented an epithelial morphology and urea and albumin production. Overall, HLCs in the MD and in 2D cultures significantly downregulated genes related to glycolysis, fatty acid and bile acid metabolism and mitochondrial function, in response to insulin; while, upon fasting and glucagon stimuli, gene expression was upregulated. Importantly, for most genes, HLCs response to insulin and fasting was at least twice more accentuated in the MD than in 2D cultures, probably due to higher inoculum and media flow in MDs that better resembles in vivo microenvironment. This work resulted in functional and metabolic responsive HLCs that may provide a cell communication platform for research.

O4

Macro-structured polylactic acid scaffolds for osteogenic regeneration

Milda Alksne[1], Migle Kalvaityte[1], Egidijus Simoliunas[1], Virginija Bukelskiene[1], Daiva Baltriukiene[1]

[1]Institute of Biochemistry, Life Sciences Center, Vilnius University, Sauletekio Ave. 7, LT-10223 Vilnius, Lithuania

Abstract. Tissue engineering is an emerging multidisciplinary field that applies the principles of engineering, material science, medicine, cell and molecular biology to create a functional artificial tissue or organ that can be transplanted into the human body. Cells, scaffolds and bioactive molecules are generally referred as the tissue engineering triad, the key components of engineered tissues. As a concequence, the ability to direct cells into a lineage-specific differentiation just by scaffold mechanical properties and geometric surface topography is one of the main tissue engineering goals. Therefore, topography of the scaffold is one of the most important factors defining the quality of artificial bone.

It is known that micro- and nano-topography of surfaces modulates cellular characteristics such as alignment and osteogenic stem cell differentiation. However the production of these scaffolds is expensive and requires high precision. Though, little is known about larger than cell diameter surface topography patterns (macro-structured) impact on stem cells fate, which could simplify production of scaffolds for bone tissue engineering. For this reason, we investigated polylactic acid (PLA) macro-pattern effect on rat dental pulp stem cells (DPSC) behaviour and osteogenic commitment.

For this study, two types of scaffolds were 3D printed– wavy (consisted of 188 µm joined threads) and porous (consisted of 500 µm threads, arranged in the woodpile manner, forming 300 µm diameter pores). Results showed that selected PLA macro-topography geometries influenced different pattern-dependent DPSC alignment and morphology which could be associated with enhanced cell proliferation and subsequent osteogenic differentiation. Moreover, tested macro-structured PLA scaffolds even without additional factors induced ALP activity, expression of osteogenesis-related genes and ECM mineralisation in DPSC. Thus, tested PLA scaffolds macro-topographies could be promising approach for cheaper and faster production of scaffolds for artificial bone tissue.

O5

Bovine preimplantation embryos at the single cell level: An accurate model for human reproductive approaches?

Nicolás M. Ortega[1] , Virpi Ahola [2], Pankaj Kumar [1] , Alice Jouneau [3] , Véronique Duranthon [3] & Fredrik Lanner [1]

[1]*Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden.*
[2]*Ming Wai Lau Centre for Reparative Medicine, Karolinska Institutet, Hong Kong, China.*
[3]*Biologie du Développement et de la Reproduction, INRA, Jouy en Josas Cedex, France.*

Abstract. The Lanner laboratory has presented a comprehensive single cell transcriptional road-map of early human embryo development and are now initiating functional gene studies using CRISPR genome editing in the early embryo. Considering the shortage of surplus human embryos donated for research, it is feasible to obtain cow ovaries from healthy animals that entered the food chain. Physiological and phylogenetic similarities, as well as readily efficient protocols available from oocyte maturation, fertilization and embryo production in vitro, encourage the use of these animal models in reproductive approaches.
In human, cells undergo an intermediate state of co-expression of lineage-specific genes, followed by a concurrent establishment of the trophectoderm (TE), epiblast EPI), and primitive endoderm(PE) lineages, which coincide with blastocyst formation. Another elusive facet of early

human development is X chromosome dosage compensation. Similar insights into the early bovine development are severely limited.
We have performed Single-cell expression analyses in individual blastomeres collected from preimplantation bovine embryos at different developmental stages from morula to expanded blastocyst. We have found top differentially expressed genes elucidating how lineage specification and pluripotency is controlled in the early bovine embryo, as well as how X chromosome dosage compensation occurs in this specie. We are complementing complement our findings with genome editing in bovine embryos by generating knock out (KO) embryos of different target genes to study their effects the in the early preimplantation embryo. We aim to translate the gained knowledge from our bovine experiments to the human embryo. We also expect to offer theoretical support for efficient derivation and culture of bovine embryonic stem cells (bESCs) in their respective human primed and naïve stem cell states.

O6

Application of the comet assay on 21-days old spheroids developed from HepG2/C3A cells

Martina ŠTAMPAR[1], Helle FRANDSEN[2], Adelina ROGOWSKA-WRZESINSKA[2], Krzysztof WRZESINSKI[3], Metka FILIPIČ[1], Bojana ŽEGURA[1]

[1] *Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Vecna pot 111, 1000 Ljubljana, Slovenia*
[2] *Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, 5230 Odense, Denmark*
[3] *CelVivo IVS 3D structures, Middelfartvej 469, 5491 Blommenslyst, Denmark*

Abstract. Safety testing of chemicals and products is at the forefront of the European legislation and REACH programme. In this respect, 3D in vitro cell-based systems are gaining importance as they more realistically mimic in vivo cell behaviour and provide more predictive results for the in vivo conditions compared to traditional 2D monolayer cultures.
The aim of our study was to develop an approach for testing genotoxic activity of chemicals on 21-days old spheroids developed from human hepatoma (HepG2/C3A) cells using dynamic bioreactor (CelVivo BAM/bioreactor) system under controlled conditions. For the determination of DNA damage, the comet assay, which is a sensitive technique for detection of DNA strand breaks, was applied. Cell spheroids were prepared by seeding HepG2/C3A cells onto AggreWell™400 plates (Stemcell Technologies) and incubated overnight.

Subsequently the cell aggregates were selected based on similarities in compactness, size as well as roundness and were transferred to bioreactors and cultured for 21 days. Twenty-one day old spheroids were then exposed for 24 and 96 hours to non-cytotoxic (measured by ATP assay) concentrations (20 and 1 µM, respectively) of indirect-acting model genotoxic compound, polycyclic aromatic hydrocarbon benzo(a)pyrene, which needs metabolic activation for its genotoxic activity. After the exposure, the suspension of viable cells from the spheroids was obtained by the combination of mechanical degradation and enzymatic (trypsin) digestion and the protocol for the comet assay on 3D was developed and optimized.
Altogether, the results demonstrated that the applied methodological approach proved to be very sensitive for detection of DNA damage induced by genotoxic compounds, suggesting that 21-day old spheroids could serve as an improved model for genotoxicity testing not only after short but also after prolonged exposures to very low concentrations of genotoxic compounds.

Acknowledgements: This study was supported by STSM grant to Martina Štampar from COST Action 16119 and by Slovenian research agency: Program P1-0245 and Grant to young investigator MŠ.

O7

Modelling the neuropathology of mucopolysaccharidosis type II with disease-specific human induced pluripotent stem cells

Julianna Kobolák[1], Kinga Molnár[2], Eszter Varga[1], István Bock[1], Bálint Jezsó[2], Annamária Téglási[1], Maria Lo Giudice[1], Norbert Varga[3], Lajos László[2], András Dinnyés[1,4]

[1]BioTalentum Ltd., Gödöllő H-2100; Hungary;
[2]Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest H-1117; Hungary;
[3]Department of Metabolic Diseases, Heim Pál Children's Hospital, Budapest H-1089; Hungary;
[4]Molecular Animal Biotechnology Laboratory, Szent István University, Gödöllő H-2101; Hungary

Abstract. Mucopolysaccharidosis II (MPS II) is a lysosomal storage disorder with progressive multisystem involvement and limited life expectancy, caused by impaired function of the iduronate 2-sulphatase (IDS) enzyme. Knowledge about its neuropathology is limited due to the unavailability of human material, although the neural symptoms are currently incurable. Therefore, our aim was to establish an in vitro human model to study its central nervous system-related pathomechanism and to provide a platform to screen alternative therapeutic products. The disease phenotype was confirmed in the established MPS II-iPSC lines and their differentiated counterparts by IDS enzyme activity and quantitative glycosaminoglycan assay. In the presence of mitogens, MPS II NPCs showed significantly decreased self-renewal capacity, although, their cortical neuronal

differentiation potential was similar to that of healthy controls. Additionally, major structural alterations in the ER and Golgi complex, accumulation of storage vacuoles, and increased apoptosis were observed in the MPS II samples. The disease-specific phenotype was more pronounced in GFAP+ astrocytes, with increased LAMP2 expression but unchanged in their RAB7 compartment. Based on these finding we hypothesise that lysosomal membrane protein carrier vesicles have an initiating role in the formation of storage vacuoles. A novel human MPS II disease model was established which recapitulates the in vitro neural phenotype of the disorder. Our system provides unlimited amount of disease-relevant cell types and offers a good platform for further study of MPS II pathophysiology or for drug testing and gene therapy studies.

O8

Cell communication in cardiac muscle cells

Jolanda VAN HENGEL, Jeffrey AALDERS, Natasja VAN DE VREKEN, Marleen DE GROOTE, Bieke VANSLEMBROUCK

Medical Cell Biology research group, Department of Human Structure and Repair, Faculty of Medicine and Health Sciences, Ghent University, Corneel Heymanslaan 10, building B, entrance 36, 4th floor 9000 Ghent, Belgium

Abstract. The mammalian heart is composed of a number of cell types including muscle cells and fibroblasts. Cardiac muscle cells are interconnected at their ends through their intercalated discs and are composed of intercellular junctions (gap junctions, desmosomes and area composita) essential for maintaining correct contraction of the heart. Cardiac fibroblasts produce and degrade extracellular matrix (ECM) components. The ECM acts as the structural network and signaling mediator in the heart.

We focus on the in vitro engineering of functional myocardium that mimics heart tissue for analysis of myocardial function:

- We differentiate functional cardiomyocytes from human pluripotent stem cells and study different genetic heart diseases (for instance Marfan disease) with the help of iPSC technology.
- Dysfunction may result from a complicated interaction of various cell types, thus more complex heart models are needed. Our objective is to mimic this interaction by realizing 3D co-culture of all cell types that make up the heart, including the fibroblasts that synthesize the ECM, to get a deeper understanding of this interaction.

- We conduct research with biomaterials suitable for the cultivation of cardiomyocytes and develop different ways to characterize the functionality of the cardiomyocytes.

We integrate these in vitro heart models with mouse models:

- Cells communicate with each other and with the ECM. The cell's internal cytoskeleton is physically connected via protein-protein interactions to other cells and the ECM. We have studied responsible interactor proteins (for instance alpha-catenins, beta-actin, cadherins).
- We investigate the functionality, morphology, histology, and ultrastructural features of interactor protein-null murine hearts, also we use (3D) cardiomyocytes derived from interactor protein-null stem cells
- The aim of our work is not only to develop 3D in vitro cultures but also make 3D reconstructions of intercellular junction of the intercalated discs obtained from mice. We demonstrated that volume scanning electron microscopy revealed the close relation between gap junctions and desmosomes and their spatial distribution in a 3D manner.

We co-operate with experts in various fields, including engineers, experts in biomaterials, electrophysiologists, geneticists and clinicians.

O9

Prostaglandin F2α stimulate endothelial cell network formation and the gene expression of angiogenic factors in the porcine endometrium

Agnieszka Waclawik, Piotr Kaczynski, Ewelina Goryszewska, Monika Baryla

Institute of Animal Reproduction and Food Research, the Polish Academy of Sciences, Olsztyn, Poland

Abstract. Prostaglandins are key mediators regulating female reproductive function in many mammal species, including pigs. Recently, we reported that PGF2α which was mainly known as a luteolytic factor, participates in the pregnancy establishment by promoting vascular endothelial growth factor synthesis and secretion, as well as increasing the expression of genes involved in embryo-maternal communication in porcine endometrium. As the endoemtial PGF2α receptor expression is elevated during peri-implantation period, the aims of the present study were to determine the effect of PGF2α on the ability of endothelial cells to form capillary-like structures and on angiopoietin 1 and 2 (ANGPT1, ANGPT2) and fibroblast growth factor 2 (FGF2) genes expression in the porcine endometrium.

Endometrial explants collected from gilts on day 12 of the estrous cycle (n=5) were incubated with PGF2α (100 nM, 1 μM) or vehicle for 24 h at 37 °C in

a humidified atmosphere (95% air and 5% CO2). After incubation, explants were snap-frozen in the liquid nitrogen. Total RNA was isolated from cells and tissues using phenol-chloroform extraction. The expression of ANGPT1, ANGPT2

and FGF2 genes was analyzed by real-time RT-PCR method. Effect of PGF2α (100 nM, 1 μM) on endothelial porcine endothelial cell line was studied using endothelial cell network assay.
PGF2α (1 uM) stimulated the expression of ANGPT1 and FGF2 genes ($p<0.05$) in endometrial explants. The expression of ANGPT2 gene was greater in endometrial explants treated with 100 nM PGF2α ($p<0.05$). Using porcine endothelial cell line, we demonstrated that PGF2α stimulated formation of capillary-like structures: the number of meshes, total mesh area and their mean size and on the number of branches and their total length. This effect was diminished by using MEK kinase inhibitor.
Our results indicate that, PGF2α regulates expression of angiogenic factors in the endometrium and may be involved in angiogenesis acting through MEK-MAPK signaling pathway during peri-implantation period.

This study was supported by National Science Centre (2012/05/E/NZ9/03493) and basic grant of IARFR PAS.

O10

3D cell rearrangement promotes DNA hypomethylation and maintains high plasticity of epigenetically erased adult somatic cells

Sharon Arcuri[1], Elena FM Manzoni[1], Sergio Ledda[2], Fulvio Gandolfi[3] and Tiziana AL Brevini[1]

[1]Center for Stem Cell Research, Department of Health, Animal Science and Food Safety, University of Milan, Italy
[2]Laboratory of Experimental Embryology, Department of Veterinary Medicine, Università degli Studi di Sassari, Italy.
[3]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy , University of Milan, Italy

Abstract. Embryo development and cell specification are driven by complex epigenetic mechanisms, that lead to a gradual loss of potency 1 and a progressive restriction in cell options. The process is reversible and cell reacquisition of a high plasticity state may be accomplished through biochemical and biological manipulations that are able to reactivate hypermethylated genes. In previous studies, we used the epigenetic modifier 5-azacytidine (5-aza-CR) to interfere with DNA methylation, erase cell specification and induce a transient pluripotent state. Here, we investigate whether the combination of epigenetic erasing to a new method that uses polytetrafluoroethylene (PTFE), a non-reactive hydrophobic synthetic compound, to produce an easy to generate and efficient micro-bioreactor, may influence global DNA methylation, boost pluripotency gene transcription and maintain long-term high plasticity. To this purpose, cells were resuspended in 30 µl of PTFE drops,

erased with 1μM 5-aza-CR and cultured in ESC medium for 28 days at 37°C in 5% CO2 in air (group A). In control experiments cells were cultured in monolayers and no resuspension in PTFE microbioreactor was carried out (group B). DNA methylation was assessed using a Global DNA Methylation ELISA Kit; CELL BIOLABS) according to the manufacturer's protocol. Expression of the pluripotent related genes, OCT4, NANOG, SOX2, REX1, was studied along the culture and at day 28. The results obtained demonstrated that group A displayed a significantly higher decrease in global DNA methylation, when compared to group B cells. Both A and B systems led to the onset of the pluripotency-related genes expression. However, while B cells turned it down by day 7, group A cells maintained it for the entire length of the experiment. In conclusion, data obtained demonstrate that epigenetic erasing coupled with a correct 3D rearrangement result in the induction and steady maintenance of a pluripotent state in adult somatic cells.

Supported by Carraresi Foundation and Ca16119

O11

Mechanical and biological activity on properties of new bone cement

Furtos G[1], Mirica IC[2], Naghiu MA[3], Declercq H[4], Gorea M[3], Prejmerean C[1], Tomoaia-Cotisel M[3]

[1]Department of Dental Materials, Raluca Ripan Institute of Research in Chemistry, Babes-Bolyai University, Cluj-Napoca, Romania
[2]Department of Oral Rehabilitation, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[3]Department of Chemical Engineering, Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University, Cluj-Napoca, Romania
[4]Department of Basic Medical Sciences, Tissue Engineering Group, Ghent University, Ghent, Belgium

Abstract. The aim of the present study was to obtain and to investigate nano forsterite and nano forsterite biocomposites for biomedical application. New self-curing forsterite biocomposites were obtained by mixing nano forsterite powder (5, 15, 30, 50, 70 % wt.) with 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)-phenyl]propane (bis-GMA) and triethyleneglycol dimethacrylate (TEGDMA) monomers. The new nano forsterite biocomposites were investigated for mechanical properties: compressive strength (CS) (143-147.12 MPa), compressive modulus (CM) (1.67-2.75GPa). Increases of CS with increasing amount of forsterite were observed up to 50 % wt. The highest CM values were registered for 70 % wt. and a direct correlation between the forsterite volume fraction (%) was observed. SEM

micrographs revealed the morphology of surface of fractured biocomposites after CS test. AFM images showed that the growth of the hydroxyapatite layer occurs with a preferred orientation on the surface of forsterite biocomposites after immersion in SBF. Incorporation of nano forsterite in the polymer matrix (bis-GMA/TEGDMA) did show osteoblast adhesion and proliferation was improved on nano forsterite biocomposites.

O12

Mesenchymal stem cells modulate the inflammatory response of mammary epithelial cells

Iftach Schouten[1,2], Nahum Shpigel[1] and Sharon Schlesinger[2]

[1]*Koret School of Veterinary Medicine*
[2]*Department of Animal Sciences, Robert H. Smith Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem*

Abstract. Mastitis, or inflammation of the mammary gland is a common disease among dairy cattle, in which it poses a major economic problem worldwide, due to losses in milk revenues and increased mortality. To date, a satisfactory treatment for Mastitis has yet to be found. Mesenchymal stem cells (MSCs) are multipotent progenitor cells, which can be found in most bodily tissues. When isolated, MSCs retain many therapeutic qualities and immunomodulatory properties. These properties have crowned MSCs as prime candidates for the treatment of many inflammatory diseases. Nevertheless, a cellular treatment of Mastitis using MSC transplantation has not been reported. As part of an ongoing research, determined to decipher whether MSCs are suitable for the treating Mastitis, we aim to investigate the effect of MSCs on the inflammatory response of EPH4 cells, a cell line of mammary epithelial cells in co-culture. We show that MSCs modulate the EPH4 inflammatory response, and that this effect is cell contact dependent. Furthermore, we find that MSCs form Tunneling Nanotubes (TNTs) and utilize them in order to deliver vesicles to EPH4 cells. Also, a

unidirectional mitochondria transfer from EPH4 cells to MSCs along MSC derived TNTs was recorded. Finally, when plated upon an EPH4 monolayer, MSCs form elaborate three-dimensional structures. In conclusion, these results point toward a unique interaction pattern between MSCs and EPH4 cells and signify an encouraging first step towards a MSC based treatment for Mastitis

P1

Pro-angiogenic effect of fibrin hydrogel functionalization with the T1 sequence from CCN1

J.C. Loureiro[1,2,3,4], A.L. Torres[1,2,3], T. Neto[1,2,3,4], P. Aguiar[1,2], C.C. Barrias[1,2,3], M.T. Pinto[1,5], and I.F. Amaral[1,2,4]

[1]3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto (UPorto), Portugal.
[2]INEB – Instituto de Engenharia Biomédica, UPorto, Portugal.
[3]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, UPorto, Portugal.
[4] FEUP - Faculdade de Engenharia, UPorto, Portugal.
[5]IPATIMUP - Institute of Molecular Pathology and Immunology of UPorto, Portugal.
iamaral@ineb.up.pt

Abstract. Hydrogel matrices with angiogenic properties are much desirable for therapeutic vascularization strategies, namely to provide vascular supply to ischemic areas, transplanted cells, or bioengineered tissues. Here we report the pro-angiogenic effect of fibrin (Fb) functionalization with the T1 sequence from the angiogenic inducer CCN1, foreseeing its use in the injured brain and spinal cord. Fb functionalization with 40 μM of T1 peptide effectively improved cellular sprouting of human brain microvascular endothelial cells (hCMEC/D3) from microcarrier beads in the absence of VEGF, without impacting either the viscoelastic properties of Fb, or the cell viability and proliferation of hCMEC/D3 cultured within the functionalized hydrogel. The pro-angiogenic effect of immobilized T1 was found to be

potentiated in the presence of VEGF and partially mediated through α6β1 integrin. The tethering of T1 also enhanced sprouting of human cord blood-derived outgrowth endothelial cells (OEC). Still, to elicit such effect, a higher input T1 concentration was required (60 μM), in line with the lower protein levels of α6 and β1 integrin subunits found in OEC comparing to hCMEC/D3 prior to embedment in Fb gel. Finally, the ability of T1-functionalized Fb in inducing cappilary invasion in vivo was assessed using the CAM assay, which evidenced a significant increase in the number of newly formed vessels at sites of implantation of T1-functionalized Fb, in the absence of soluble angiogenic factors. Overall these results demonstrate the potential of T1 peptide-presenting gels for use in therapeutic vascularization approaches. Considering T1 neurite-extension promoting capability and pro-angiogenic properties, T1-functionalized Fb gels are particularly promising for application in the injured CNS.

Acknowledgments: projects NORTE-01-0145-FEDER-000008 and NORTE-01-0145-FEDER-000012, financed by NORTE 2020, FEDER funds through COMPETE 2020 – POCI; project "Institute for Research and Innovation in Health Sciences" (POCI-01-0145-FEDER-007274), and Grant Prémio Melo e Castro 1068-2015 from Santa Casa da Misericórdia de Lisboa.

P2

How CCL8 affect cells from porcine reproductive organs

Aneta Andronowska, Krzysztof Jan Witek, Aleksandra Złotkowska

Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

Abstract. Chemokines, besides its known chemotactic role, have also been implicated in a number of reproductive events, such as ovulation, embryo implantation, parturition or endometriosis. CCL8 also known as MCP-2, is produced by monocytes and macrophages, and acts through receptors CCR1, CCR2 and CCR5. Our earlier study revealed expression of CCL8 and it receptors in the porcine endometrium and corpora lutea during both the estrous cycle and pregnancy, and their proteins are localized in endometrial luminal epithelial and glandular cells, luteal cells and in vascular endothelium. The aim of this study was to evaluate the influence of CCL8 on cells derived from porcine endometrium, corpus luteum and umbilical cord (immortalized swine umbilical endothelial cells, line G1410).
CCL8 stimulated proliferation and migration ($p<0.05$) of endometrial epithelial cells but decreased it adhesion ($p<0.05$). Scratch assay revealed that CCL8 stimulate endometrial epithelial cells movement (% of gap closure) and was the most potent of CC- chemokine. Proliferation of endometrial stromal and endothelial ($p<0.05$) as well as G1410 ($p<0.05$) cells was also increased after CCL8 stimulation. Chemotactic effect of CCL8 was observed in

endometrial endothelial cells, however CCL8 did not affect tube- like structures formation of endothelial cells from endometrium, corpus luteum or umbilical cord. To conclude, our observations revealed tissue- and cell- specific role of CCL8 in porcine reproductive system.

P3

T regulatory cell-dependent attenuation of neutrophils is crucially important for the attenuation of cisplatin-induced acute renal failure

Arsenijevic A[1], Simovic Markovic B[2], Gazdic Jankovic M[1], Lukic ML[1] and Volarevic V[2]

[1]*Department of Genetics, Faculty of Medical Sciences Universuty of Kragujevac*
[2]*Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Serbia*

Abstract. Background. T regulatory cells (Tregs) protect against cisplatin (CDDP)-induced nephrotoxicity while neutrophils may have protective or aggressive role, in dependence of cytokine production. In this study, we analyzed the cross-talk between renal Tregs and neutrophils in CDDP-injured kidneys.
Materials and method. Cisplatin (16mg/kg body weight) was injected in C57Bl/6 mice to induce acute kidney injury (AKI). Cyclophosphamide (CY)-induced depletion of Tregs was used to demonstrate the importance of Tregs for alleviation of neutrophil-driven CDDP-induced AKI. Biochemical, histological analysis, ELISA, real-time PCR, flow cytometry and intracellular staining of renal-infiltrated neutrophils were used to determine the extent of AKI.
Results. CDDP caused significant renal dysfunction as reflected by a marked elevation of serum urea and creatinine, severe tubular epithelial cell injury, increased serum levels of IFN-γ and IL-17 and decreased serum levels of

IL-10. Accordingly, massive presence of IFN-γ and IL-17 producing neutrophils and reduced presence of IL-10-producing neutrophils and Tregs were observed in CDDP-treated mice. In vitro, Tregs, primed by TLR-2 activated dendritic cells (DCs), showed better immunosuppressive effects against neutrophils and promoted neutrophil conversion from IFN-γ and IL-17-producing into IL-10-producing cells. TLR-2 activated DCs managed to attenuate CDDP-induced AKI, but depletion of Tregs completely diminished DC-based nephroprotective effects by significantly increasing total number of renal-infiltrating IFN-γ and IL-17-producing neutrophils and decreasing presence of IL-10-secreting neutrophils.
Conclusions: Tregs-dependent attenuation of neutrophils is crucially important for the attenuation of CDDP-induced acute renal failure.
Keywords: cisplatin; acute renal injury; Tregs; neutrophils

P4

Proximity Ligation Assay demonstration of the interaction between Human papillomavirus type 16 E6 and E7 oncoproteins with the DNA damage nuclear sensor p53-binding protein 1 in an in vitro reconstructed 3D epithelium

Diletta Francesca Squarzanti, Rita Sorrentino, Andrea Chiesa and Barbara Azzimonti

University Piemonte Orientale (UPO), School of Medicine, Department of Health Sciences

Abstract. INTRODUCTION. Human papillomaviruses (HPV) are very well known for their ability to infect skin and mucosa. Even if research progresses and vaccines have reduced their incidence, they still cause oropharyngeal and anogenital malignancies, mainly through HPV16 E6E7 oncoproteins.
Host cells generally respond to DNA damage thanks to peculiar DNA damage sensors. When epithelial cells are infected and induced to proliferate by HPVs, the DNA Damage Response (DDR) isn't inactivated by the virus; no explanation for this has been formulated yet.
When HPV16 infects an epithelium, E6 and E7 oncoproteins bind and degrade/inactivate p53a and pRb both through a LXXLL domain, but their role in the DDR activation is not well known.
EXPERIMENTAL MODEL. We generated an in vitro 3D HPV16-E6E7 infected epithelium, and looked for the presence of HPV16E6E7 viral oncoproteins and of the double strand

breaks (DSB) sensor 53BP1; we then made a colocalization immunofluorescent analysis.
Since HPV16 oncoproteins both interact with LXXLL containing proteins, we analyzed 53BP1 BRCT2 motif and the possible binding among these proteins via the in situ PLA assay both in monolayer in CaSki and E6E7HPV16 keratinocytes and in the 3D model.
RESULTS. E6E7HPV16 induce a 53BP1 increase in nuclear foci in the reconstructed infected epithelium. E6 and E7 co-localise with 53BP1; 53BP1 BRCT2 domain also harbour a LKVLL sequence; E6 and E7 both interact with 53BP1.
CONCLUSION. HPV16 oncoproteins are able to overcome host's cell DDR strategies.

P5

A step by step development of an oviduct-on-a-chip model for better biomimicking in vivo early embryo development

Bart M. Gadella

Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 104 3584 CM, Utrecht, The Netherlands

Abstract. Millions of children have been conceived using assisted reproductive technologies (ART). Most of the research in the field of ART only focuses on increasing the low efficiency of obtaining and maintaining pregnancy or live birth, whereas the long-term impact on the health of the resulting child has been relatively neglected. Animal models have highlighted that in vitro embryo culture is not exactly mimicking embryo development in the female genital tract and also has effects after embryo transfer on subsequent offspring development and health. In vivo, the oviduct hosts the first embryo developmental period coinciding with a complete reprogramming of its (epi)genome in preparation for the reacquisition of (epi)genetic marks. In this context, an oviduct-on-a-chip platform was tested as a more biomimic model that may serve to better understand the mechanisms related to epigenetic and genetic reprogramming. The oviduct-on-a-chip device was found to support the conditioning of an apical fluid compartment that allowed exclusive monospermic fertilization (60 % efficiency) and more physiological (in vivo-like) zygote genetic reprogramming of these fertilized oocytes than under

conventional IVF. In the development of the device, specific issues such as release of toxic components from the biofabricated materials and affinity of these materials for bioactive components such as steroid should be considered.

P6

The Hyppo signaling pathway and TAZ dependent SMAD shuttling control pluripotency of PTFE encapsulated adult somatic cells

Tiziana AL Brevini[1], Sharon Arcuri[1], Georgia Pennarossa[1], Sergio Ledda[2] and Fulvio Gandolfi[3]

[1]Center for Stem Cell Research, Department of Health, Animal Science and Food Safety, University of Milan, Italy
[2]Laboratory of Experimental Embryology, Department of Veterinary Medicine, Università degli Studi di Sassari, Italy.
[3]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy , University of Milan, Italy

Abstract. We previously demonstrated that epigenetic erasing, coupled with a correct 3D rearrangement, result in the induction and steady maintenance of a pluripotent state in adult somatic cells. In order to better understand the mechanisms linking 3D cell rearrangement and the maintenance of high plasticity we here investigate the involvement of the Hippo signaling mechano-transduction pathway and evaluate the interaction with the crucial pluripotency regulator SMAD family member 2 (SMAD2). Cells were resuspended in 30 µl of PTFE drops, erased with 1 µM 5-aza-CR and cultured in ESC culture medium for 28 days (Group A). In control experiments cells were cultured in monolayers with no encapsulation in PTFE microbioreactor (group B). The activation of the two main Hippo signaling regulators YAP/TAZ was evaluated by Western Blot and

Immunestaining of whole cell lysate with a ReadyPrep Protein Extraction Kit (Bio-Rad). Nuclear extracts from the cells were isolated using the NXtract CelLytic NuCLEAR Extraction Kit (Sigma). The results obtained show that PTFE micro-bioreactor activates the Hippo signaling pathway to maintain high plasticity in epigenetically erased cells. In particular, fibroblasts exposed to 5-aza-CR showed TAZ nuclear accumulation both in cells A and cells B. TAZ nuclear confinement was however lost by Group B cells, with the molecule relocating to the cytoplasm, by day 7 of culture. In contrast, 3D cell confinement of Group A cells was stably maintained for the entire length of the experiments. In addition, TAZ localization was mirrored by a parallel compartmentalization of SMAD2, that lost its nuclear confinement in response to siRNA knockdown of TAZ, with loss of pluripotency marker transcription. Altogether, this suggest that 3D PTFE cell encapsulation and 3D rearrangement is able to induces and maintain pluripotency via the direct involvement of the Hippo signaling pathway, TAZ nuclear confinement and SMAD2 co-shuttling

Supported by Carraresi Foundation and CA16119

P7

The use of 3D-cultured human neonatal mesenchymal stem cell secretome for counteracting rheumatoid arthritis symptoms

Sérgio P Camões[1], Manuela M Gaspar[1], Joana S Rodrigues[1], Manuela Carvalheiro[1], Sandra Simões[1], Jorge M Santos[2], Joana P Miranda[1]

[1] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal
[2] Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências e Tecnologias Agrárias e Agro-Alimentares (ICETA), Universidade do Porto, Porto, Portugal

Abstract. Rheumatoid arthritis (RA) is an autoimmune disorder whose treatment is mostly restricted to pain management and joint destruction delay. Mesenchymal stem cells from umbilical cord tissue (UC-MSCs) have been proven to be immunomodulatory and more efficient than bone marrow-derived MSCs in the remission of arthritic manifestations in vivo. These effects are essentially mediated by cellular communication through a paracrine mechanism, which makes UC-MSC secretome promising as an ATMP. Herein, we aimed at demonstrating the viability of applying a 3D-culture-based UC-MSC priming strategy to improve the efficacy of the resulting secretome for RA treatment. A proteomic analysis was performed to both, media conditioned by UC-MSC cultured either under 2D (CM2D) or 3D (CM3D) conditions. The analysis of relevant trophic factors confirmed distinct secretome profiles in terms of therapeutic potential. Whereas CM3D was characterised by a

prevailing expression of anti-inflammatory cytokines (e.g. IL-10 and LIF) and trophic factors involved in different mechanisms leading to tissue regeneration (e.g. PDGF-BB, FGF-2, I-309, SCF and GM-CSF); CM2D presented relatively higher levels of IL-6, MCP-1 and IL-21, with recognized pro-inflammatory roles in joint disease and pleiotropic effects in RA progression. Accordingly, CM3D promoted a ~1.5-fold increase in chondrocyte migration capacity when compared to CM2D; and a ~2-fold increase in GAG induction by CM2D when compared to CM3D. Finally, the evaluation of arthritic manifestations in vivo, using a rat adjuvant-induced model for arthritis (AIA), suggested a significantly higher therapeutic potential of CM3D over CM2D and even UC-MSCs. Histological analysis confirmed a faster remission of arthritic manifestations of CM3D-treated animals. Overall, the results show that UC-MSC CM3D is a viable and better strategy than direct UC-MSC administration for counteracting AIA-related signs, representing a novel MSC-based cell-free treatment for RA.

P8

Characterization of oviductal extracellular vesicles from early metestrus sows

Shuai Chen[1], Inga Läzer[1], Sergio E. Palma-Vera[1], Fan Liu[2], Marcus Frank[3], Jennifer Schoen[1]

[1]Institute of Reproductive Biology, Leibniz Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany.
[2]Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Robert-Rössle-Str. 10, 13125 Berlin, Germany.
[3]Medical Biology and Electron Microscopy Centre, Rostock University Medical Center, Strempelstraße 14, 18057 Rostock, Germany.

Abstract. Establishment of pregnancy requires an effective exchange of signals between the embryo and the female reproductive tract. Extracellular vesicles (EVs) within the oviductal fluid (OF) have recently been identified as messengers in embryo-maternal interaction. Therefore, we aimed to characterize porcine EVs from OF during early metestrus and initially investigate their effect on early embryonic development. OF was collected from sows (n=4) two days after ovulation. Polyethylene glycol (PEG) precipitation and washing by ultracentrifugation was used to isolate EVs. EVs were visualized by transmission electron microscopy (TEM) and their protein cargo was characterized by mass spectrometry. Later, in vivo fertilized zygotes (n=54) were co-cultured with EVs (3-6x1010/ml) and monitored using a time-lapse system. Proteomic analysis revealed in total 1360 proteins, including exosomal membrane and

adhesion markers, molecules enriched in microvesicles, and EV synthesis and trafficking molecules, reflecting the presence of different EV subpopulations, as confirmed by TEM. A majority of the top 20 most abundant proteins were associated with reproductive functions as fertilization and early embryo development (e. g. OVGP1, HSP90A and HSPA8), sperm reservoir and embryo adhesiveness (e. g. ANXA1, 2, 4, 5), and oviduct-sperm interactions during metestrus (MYH9, ACTB). In the co-culture experiment cleavage rate was 100% in both EV and control group. Also blastocyst (80-90%) and hatching rates (15-22%) were comparable. No difference in the temporal course of embryonic development could be observed. However, blastocysts co-cultured with EVs had fewer nuclei. To conclude, even though metestrus oviductal EVs were loaded with proteins associated to reproductive processes, in our study no obvious beneficial effects of EVs on porcine embryo development could be observed. In view of decreased numbers of nuclei, a negative effect of residual PEG on embryo development can't be ruled out.

P9

Gal-3 promotes TLR-2-dependent activation of IDO1/KYN pathway in renal DCs resulting in increased expansion of immunosuppressive Tregs

Djokovic B[1], Simovic Markovic B[1], Gazdic Jankovic M[2], Arsenijevic N[1], Lukic ML[1] and VolarevicV[1]

[1]Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac
[2]Faculty of Medical Sciences, University of Kragujevac, Department of Genetics

Abstract. Background. Galectin 3 (Gal-3) regulates migration, proliferation and activation of renal dendritic cells (DCs) and T regulatory cells (Tregs) which protect against cisplatin (CDDP)-induced nephrotoxicity. Since molecular mechanism responsible for the cross-talk between renal DCs and Tregs in CDDP-injured kidneys is still unknown, we analyzed the importance of Gal-3 for this interaction.
Materials and method. Dendritic cells were isolated from wild type (WTDCs) and Gal-3 deficient mice (Gal-3-/-DCs) by magnetic cells sorting. Pam3CSK4 was used for activation of Toll-like receptor (TLR)-2 in WTDCs (WTDCsPam3CSK4) and Gal-3-/-DCs (Gal-3-/-DCsPam3CSK4). Newly synthesized selective inhibitor of Gal-3 (Davanat) and 1-Methyl Tryptophan (1-MT) were used for pharmacological inhibition of Gal-3 and Indoleamine 2,3-dioxygenase-1 (IDO1) in TLR-2-primed WTDCs (WTDCsPam3CSK4+Davanat and WTDCsPam3CSK4+1-MT).

Results. Genetic deletion as well as pharmacological inhibition of Gal-3 remarkably reduced TLR-2-dependent secretion of Kynurenine (KYN) in renal DCs and diminished their capacity to maintain immunosuppressive phenotype of Tregs in vitro and in vivo. WTDCsPam3CSK4+Davanat and Gal-3-/-DCsPam3CSK4 were not able to prevent transdifferentiation of immunosuppressive Tregs in inflammatory Th1 or Th17 cells. Additionally, Tregs generated by WTDCsPam3CSK4+Davanat or Gal-3-/-DCsPam3CSK4 showed reduced immunosuppressive effects in vitro.
Conclusions. Gal-3 promotes TLR-2-dependent activation of IDO1/KYN pathway in renal DCs resulting in increased expansion of immunosuppressive Tregs.
Keywords. Gal-3; IDO-1; TLR-2; dendritic cells, T regulatory cells

P10

The role of extracellular vesicles as indicators of cellular defense mechanisms during environmental stress in bovine granulosa cells

Samuel Gebremedhn[1], Ahmed Gad[2,3], Jozef Laurincik[2,4], Radek Prochazka[2], Hoda Samir Aglan[1],Michael Hoelker[1], Karl Schellander[1], Dawit Tesfaye[1]

[1]*Institute of Animal Science, Animal Breeding and Husbandry Group, University of Bonn, Bonn, Germany*
[2]*Laboratory of Developmental Biology, Institute of Animal Physiology and Genetics of the Czech Academy of Sciences, Liběchov, Czech Republic*
[3]*Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt*
[4]*Constantine the Philosopher University in Nitra, Nitra, Slovakia*

Abstract. Heat stress (HS) is one of the key environmental stressors that cause infertility in dairy cows. HS is reported to be associated with ovarian dysfunctionality. Exposure of bovine granulosa cells (bGCs) to HS leads to massive deregulation of apoptosis and steroidogenesis-related genes. However, the impact of HS on bGCs cellular and extracellular miRNA profile is not fully understood. Here, we aimed to determine the impact of HS on the expression of miRNAs in bGCs and the corresponding EVs. For this, bGCs were aspirated from smaller follicles and bGCs were cultured in DMEM/F-12 media supplemented with 10% exosome-depleted FBS. For the first 24 hours, cells were incubated at 37OC and the next 24 hours cells were grouped as control

(37OC) and stressed (42OC). The spent culture media was subjected to EVs isolation using ExoQuick-TC and molecular and morphological characterizations. MiRNA enriched total RNA was isolated from cells and EVs using miRNeasy mini kit (Qiagen) and Exosomal RNA Isolation Kit (Norgen), respectively. RNA samples were subjected to miRNA library preparation and NGS (QIAGEN Genomic Services). Differential expression analysis of miRNAs in bGCs and EVs subjected to HS was determined using EdgeR. The impact of HS on the expression of stress-related genes, cell proliferation, total oxidized protein and, ROS accumulation were performed. Results showed that the expression of heat shock proteins genes (HSP70 and HSP90), oxidative stress-related genes (NRF2 and SOD) and ER stress-related genes (GRP78 and GRP94) was induced by HS. Moreover, HS caused higher accumulation of ROS and total oxidized proteins resulting in reduced cell viability. HS did not lead to significant differences in the size and morphology of released EVs. However, more EVs were released from heat-stressed bGCs. Sequence analysis detected 315 and 310 known miRNAs in stressed and unstressed cells, respectively. Differential expression of miRNAs revealed 3 and 4 miRNAs to be down and up-regulated, respectively. Similarly, 2 miRNAs and 4 miRNAs were down and upregulated in EVs released from stressed bGCs, respectively. Interestingly, miR-1246, which was upregulated in stressed cells, was also enriched in the corresponding EVs. This could suggest the potential role of EVs as indicators of the cellular stress condition in bGCs.

P11

Genetic inhibition of Atg5 in mesenchymal stem cells abrogates their capacity to suppress NKT cells in acute liver injury

Gazdic M[1], Simovic Markovic B[2], Misirkic M[3], Arsenijevic N[2], Lukic ML[2], Trajkovic V[3], Volarevic V[2]

[1]Department of Genetics, Faculty of Medical Sciences Universuty of Kragujeva
[2]Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences,University of Kragujevac,Serbia
[3]Institute of Microbiology and Immunology, School of Medicine, University of Belgrade

Abstract. Background. Therapeutic potential of mesenchymal stem cells (MSCs) in acute liver failure is mainly based on its release of immunomodulatory factors. Since the effect of autophagy inhibition on the immunomodulatory characteristics and the cellular survival in the inflammatory microenvironment in acute liver injury is not known, we examined whether genetic inhibition of autophagy modulates hepatoprotective capacity of MSCs.
Materials and method. Genetic inhibition of autophagy in MSCs was conducted with siRNA targeting Atg5 (MSCAtg5). After transfection, MSCsAtg5 were allowed to grow 24 hours before used for experiments. The mouse model of fulminant hepatitis was induced by application of alpha-galactosylceramide (α-GalCer, 50 μg/kg).
Results. Serum alanine transaminase (ALT) and aspartate transaminase (AST) levels were significantly higher in α-GalCer+ MSCsAtg5- compared to α-GalCer+MSCs-treated

animals. To examine histological changes in the liver, we subjected liver tissues to H&E staining. The necroinflammatory foci in the livers of α-GalCer+ MSCAtg5-treated mice were larger and more abundant compared with those in α-GalCer+MSC-treated animals, consisting of a mixed population of mononuclear cells and apoptotic hepatocytes. Cellular make-up of the liver revealed that MSCAtg5 treatment markedly reduced percentage of protective IL-10 producing Tregs, while favored inflammatory IL-17- and IFN-γ-producing NKT cells in the livers of α-GalCer–treated mice. On contrary to MSCs, injection of MSCAtg5 significantly increased percentage of liver-infiltrating CD11c+DCs that expressed co-stimulatory molecules CD86 and CD80 while decreased presence of IL-10 producing DCs in the liver of α-GalCer-treated mice.
Conclusion. Genetic inhibition of Atg5 in MSCs abrogates their hepatoprotective and immunomodulatory effects in acute hepatitis.
Keywords. mesenchymal stem cells; autophagy; Atg5, acute liver injury, NKT cells

P12

Expression of OCT4 and GATA4 transcription factors in rabbit MSCs after cardiogenic induction in vitro

N. Grigorova[1,*], Vachkova, E.[1], Elen Gócza[2]

[1]Department of Pharmacology, Animal Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, 6000, Stara Zagora, Bulgaria
[2]Department of Animal Biotechnology, Applied Embryology and Stem Cell Research Group, NARIC, ABC, Gödöllő, Hungary

[*]Corresponding author: Natalia Grigorova, PhD, Animal Physiology Unit, Department of Pharmacology, Animal Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria; GSM: +359 885 476 293; E-mail: n.gr@abv.bg

Abstract. As a consequence of cardiovascular diseases, 17.9 million people die annually [WHO]. Mesenchymal stem cells (MSCs) have been considered to be preferable cell types in cardiac regeneration and cell-based therapies because of their allogeneic high proliferative potential. Addressing the electrophysiological properties of the heart, rabbit is closer to human than mouse.
The current study aimed to trace changes in comparative manner of OCT4 and GATA 4 mRNA expression in rabbit embryonic fibroblasts and rabbit MSCs obtained from subcutaneous, bone marrow and pericardial tissue during cardiomyocyte differentiation in vitro.

Methods: The cardiac differentiation protocol for mouse embryonic stem cells in hanging drop was applied for two days in parallel for rabbit embryonic fibroblasts and rabbit MSCs. The best formed EBs (embryonal bodies)-like structures were collected and further cultivated in suspension for 3 days. The total mRNA was obtained prior cardiac differentiation and on the 6th day of it. SYBR Green – based real time PCR was performed to evaluate the expression of the target genes: OCT4 and GATA4. ΔΔ Ct method with GAPDH as a house-keeping gene were used for data analyses.
Results: The cultivation of MSCs in hanging drops during cardiac differentiation induced EBs formation, without any contractile activity up to the 6th day of the differentiation. The applied differentiation protocol significantly downregulated GATA4 expression in ADSCs - EBs, while in BMSCs and rabbit embryonic fibroblasts EBs both target genes were significantly upregulated.
Conclusions: The adopted cardiac differentiation protocol in hanging drops from mouse embryonic stem cells could be applied with some modifications in rabbit embryo fibroblast and bone marrow, while in ADSCs (subcutaneous and pericardial stem cells) it had negative effect. We assume that concerning a cardiomyocyte differentiation, a specific approach should be applied depending on the rabbit MSCs origin.

P13

Assessment of genotoxic activity of cyanotoxin cylindrospermopsin using human hepatoma in vitro 3D cell model

Klara HERCOG, Martina ŠTAMPAR, Metka FILIPIČ, Bojana ŽEGURA

Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Vecna pot 111, 1000 Ljubljana, Slovenia

Abstract. Cyanobacterial toxin cylindrospermopsin (CYN) is increasingly being found in surface freshwaters worldwide and is considered as an emerging environmental pollutant. It poses a potential threat to humans after chronic exposure as it has been shown to be pro-genotoxic, meaning that it has to be metabolized to induce DNA damage.

In the present study, newly developed 3D cell model from human hepatoma (HepG2) cells was for the first time used for the evaluation of genotoxic activity of CYN. HepG2 spheroids prepared by forced floating method were cultured for three days under static conditions and were subsequently exposed to non-cytotoxic concentrations of CYN (0.125, 0.25 and 0.5 µg/mL) for 72 hours. After the exposure, the suspension of viable cells was prepared from the spheroids with the combination of mechanical and enzymatic (trypsin) degradation. The influence of CYN on spheroid cell survival, division and proliferation was determined by planimetry, proliferation marker Ki67 and cell cycle analyses, while the induction of DNA double strand breaks (DSB) was evaluated with the □H2AX foci assay using flow cytometry. The results

revealed that CYN affects the growth and division of cells in HepG2 spheroids. The visual inspection and average area measurement indicated reduced spheroid growth and morphologic changes of cells in spheroids exposed to CYN, while decreased cell proliferation was determined with imunocytochemical analysis of the cell-proliferation marker Ki67. In addition, the influence of CYN on cell growth was confirmed by cell-cycle analysis using flow-cytometry. In HepG2 spheroids, CYN induced DNA DSB after 72-hour exposure confirming its genotoxic activity.
The HepG2 spheroid model provides a simple, practical and cost effective tool that can be effectively used in toxicological and environmental research, including assessment of genotoxic potential and effect-based research of various compounds including environmental toxins.

Acknowledgements: This study was supported by Slovenian research agency: Program P1-0245 and Grant to young investigator KH and MŠ.

P14

Amino acids turnover as a possible indicator of the developmental potential of human embryos after thawing

M. Jeseta[1], A. Mádr[2], J. Žáková[1], P. Ventruba[1], A. Celá[2], I. Crha[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Masaryk University Brno and University Hospital Brno, Brno, Czech Republic.
[2]Department of Biochemistry, Faculty of Science, Masaryk University Brno, Czech republic

Abstract. Metabolomic changes associated with embryo regeneration after thawing are still unknown. In future, their clarification would be the way to optimize standard thawing protocols for more effective regeneration of human embryos after vitrification. In this study, embryos were assessed immediately after thawing by routine morphological criteria and subsequently cultured for 24 hours in individual 25 µl drops of GTL medium. After this time, the embryos were assessed morphologically and selected as progressive (n=21) and nonprogressive (n=22). A total of 20 amino acids were derivatized by naphthalene-2,3-dicarboxaldehyde in the presence of sodium cyanide. Products of the derivatization were separated and detected by micellar electrokinetic chromatography with fluorescence detection. Our results showed that metabolic activity of progressive and nonprogressive human embryos was different in total amino acid turnover (sum of total amino acid consumption and production), which was significantly lower ($P<0.05$) in the

progressive embryos in comparison to the nonprogressive embryos.

Supported by CA MH CZ – DRO (FNBr, 65269705), project MSMT LTC 18059 and AZV NV18-01-00544.

P15

Aged rabbit adipose-derived mesenchymal stem / stromal cells (ASCs) recapitulate aging biomarkers and show reduced stem cell plasticity

Juliane-Susanne Jung[1], Christin Volk[1], Christina Marga[1], Alexander Navarrete Santos[2], Matthias Jung[3], and Anne Navarrete Santos[1]

[1]*Institute of Anatomy and Cell Biology, Medical Faculty of Martin Luther University, Halle (Saale), Germany*
[2]*Centre for Medical Basic Research, Medical Faculty of Martin Luther University, Halle (Saale), Germany*
[3]*Department of Psychiatry, Psychotherapy, Psychosomatic Medicine, Medical Faculty of Martin Luther University, Halle (Saale), Germany*

Abstract. The rabbit is a valuable animal model for a variety of biomedical research areas including embryology, organogenesis, and modelling of diabetes, obesity, or cardiovascular diseases. Embryo and fetal development of the rabbit are often analysed in toxicology studies and drug development. However, little is known about the application of rabbits for modelling aging and age-related disease mechanisms. Adult stem cells such as mesenchymal stem cells are affected by molecular and cellular aging mechanisms. They have the capability to self-renew and can differentiate into multiple cell types of the mesoderm germ layer. Therefore, ASCs represent a promising tool for studying age-related diseases in detail.

ASCs from young (16 weeks) and old (>108 weeks) female rabbits were successfully isolated, propagated, and characterized. Cultured primary ASCs showed the typical morphology of mesenchymal stem cells. ASCs stained positive for cluster of differentiation (CD) 105, Vimentin, Collagenase 1A and negative for CD14, CD90, CD73 demonstrating their mesenchymal origin. ASCs expressed mesenchymal stem cell markers including MYC, KLF4, CHD1, REST, KAT6A, while the pluripotency-related genes such as NANOG, OCT4, and SOX2 were not expressed. Aged ASCs showed altered protein and mRNA levels of APOE, ATG7, FGF2, and SIRT1. The adipogenic differentiation of old visceral ASCs was significant decreased compared to young visceral ASCs. Furthermore, ASCs from old rabbits showed increased levels of H3K27ac that is characteristic for a more open and accessible chromatin state. The loss of heterochromatin is one potential factor that contributes to stem cell aging.

We successfully established rabbit ASC cultures representing an in vitro model for the analysis of stem cell aging mechanisms. ASCs, obtained from old female rabbits, showed age- and source-specific alteration due to aging of the donor. Stem cell plasticity was altered with age shown by reduced adipogenic differentiation capacity.

P16

Adipose tissue-derived mesenchymal stem cells and breast cancer cells crosstalk in 3D conditions: involvement of NF-kB and HIF-1 signaling pathways

Tamara Kukolj[1], Aleksandra Jauković[1], Jana Plava[2], Marina Cihova[2], Drenka Trivanović[1], Diana Bugarski[1], Lucia Kucerova[2], Svetlana Miklikova[2]

[1] Laboratory for Experimental Hematology and Stem Cells, Institute for Medical Research, University of Belgrade, Serbia
[2] Cancer Research Institute, Biomedical Research Center, Bratislava, Slovakia

Abstract. Tumors are heterogeneous entities in which tumor cells exists in close proximity with noncancerous cells, such as mesenchymal stromal/stem cells (MSCs). Adipose tissue-derived (AT)-MSCs are considered as potentially valuable tool for breast cancer therapy, as well as breast tissue reconstruction. However, in terms of tumor microenvironment, their role is still controversial, as both pro- and anti-tumorigenic effects were described. They have the potential to significantly influence malignant progression and metastatic abilities of tumor cells. Moreover, of special importance are their interactions described and established in 3D conditions showing enrichment of tumorigenic 'stem' cells upon mammosphere formation. Increasing evidence also indicate that tumorigenic process in breast cancer is dependent on numerous inflammatory factors and signaling pathways. Regarding all these facts, our study analyzed NF-kB

and hypoxia inducible factor (HIF)-1 involvement in AT-MSCs/BCC cross-talk, since activation of inflammation and hypoxia play an important role in cancer stemness and disease progression. Special attention was directed to the BCC survival and growth in 3D system. Namely, following the incubation with conditioned medium derived from AT-MSCs or after direct coculture with AT-MSCs pretreated with specific NF-kB and HIF-1 inhibitors, relative viability of BCC in spheres was analyzed by 3D luminescence assay in real time. Along with growth analyses, BCC stemness features were detected based on OCT4, SOX-2 and NANOG protein expression by using immunofluorescence staining. Overall, collected data provide important insight into AT-MSCs/BCC communication that should be further explored in order to understand oncological risk for clinical use of AT-MSCs in breast tissue therapy.

This study was supported by Bilateral project between Slovakia and Serbia (Grant SK-SRB-2016-0031), Slovak Research and Development Agency (Grants APVV-16-0178, APVV-16-0010, VEGA 1/0271/17) and Serbian Ministry of Education, Science and Technological Development (Grant 175062).

P17

Analysis of effects of plasma treatment of beta tri-calcium phosphate on regeneration potential of periodontal ligament mesenchymal stem cells in vivo

Miletić M., Mojsilović S., Brković B., Andrić M., Milutinović-Smiljanić S., Prokić B., Škoro N., Puač N.

Faculty of Dental Medicine, Institute for Medical Research, Faculty of Veterinary Medicine, Institute of Physics, University of Belgrade

Abstract. Large bone defects caused by trauma, osteoporotic fractures, infection and tumor or cyst resection pose a great clinical and socio economic problem. This led to development of different synthetic bone grafts. Last generation of grafts relies in the use of biomaterials such as calcium phosphate ceramics alone or in combination with natural or synthetic polymers. Combination of biomaterials with mesenchymal stem cells (MSCs) has been shown to be a suitable strategy for regeneration of bone defects. Recent reported data demonstrates the ability of plasma technology to modify the physicochemical properties of biomaterial surfaces, as a potentially efficacious strategy to control and influence the response of human MSCs.

In order to assess in vivo effects of non-thermal atmospheric plasma (NAP) treatment on regenerative potential of human periodontal ligament MSCs (PDL-MSC) and beta tricalcium phosphate (bTCP) an experimental study is performed using rabbit calvaria critical size defects model. The PDL-MSC are

isolated from human periodontal ligament of normal impacted third molars, characterized and cultivated until the experiment. bTCP was treated with NAP using atmospheric pressure dielectric barrier discharge prior to the implantation. With rabbits under general anesthesia, four bone defects were made, and filled with treated/non-treated bTCP with or without PDL-MSC. After two and four weeks of healing, animals were sacrificed and samples were taken for histomorphometric analysis.

In the group with bTCP alone, the amount of the newly formed bone at two and four weeks was 3.66% and 5.01%, and with treated bTCP without cells, 4.85% and 7.76%, respectively. The amount of newly formed bone in treated/non-treated bTCP with cells was 20.70% and 19.18% at two weeks and 22.19% and 17.64% at four weeks, respectively. In addition, the amount of residual biomaterial decreased in the groups with cells compared to groups with treated/non-treated bTCP alone.

Our study indicates that the PDL-MSC enhanced bone regeneration in healing critical-size rabbit calvarial defect, but further study is needed to reveal if pretreatment of the biomaterial with NAP improves this regeneration.

P18

A comparison of the cytokine profiles between 2D and 3D cultured mesenchymal stromal cells

Milena Kostadinova, Radko Sotirov, Snejana Kestendjieva, Shina Pashova, Elena Stoyanova, Kamelya Vinketova, Ivan Bochev, Tsvetelina Oreshkova, Milena Mourdjeva

Department Molecular Immunology, Institute of Biology and Immunology of Reproduction, BAS

Abstract. Mesenchymal stem/stromal cells (MSC) cultured in spheroids have enhanced anti-inflammatory, angiogenic, and tissue reparative/regenerative effects caused by changes in their gene expression profile. This study compares the cytokine profiles of MSC obtained from adipose tissue (ASC) and umbilical cord's Wharton jelly (WJ-MSC) cultured in 2D and 3D.

Thirty-six cytokines and chemokines in cell culture supernatantes were measured by Proteome ProfilerTM Array, Human Cytokine Array Panel A. MSC panel of markers (CD29, CD72, CD90, CD45) were analyzed by FACSCalibur. Cell viability was determined by Propidium Iodide exclusion assay.

A comparison of the cytokine profiles between 2D and 3D culture revealed a substantial increase of pro-inflammatory factors in 3D ASC cultures. Our preliminary results showed more than 14 times difference for G-CSF expression in 3D compared to 2D; 6,7 times increase for IL-1ra; more than 5 times for sICAM-1; 4,5 times increase of CCL5; C5/C5a, IL-8 and SDF-1 were also increased in 3D. WJ-MSC 3D cultures also showed an elevation of pro-inflammatory

mediators/factors CCL5 and SDF-1 (~2 times), while other pro-inflammatory cytokines, such as G-CSF, IL-1ra, IL-8 remain unchanged. sICAM-1, C5/C5a, IL-1a, IL-1b, CXCL-1, G-CSF and GM-CSF remained lower in comparison to 2D culture.
A systemic reduction in vitality from 2D (~90%) to 3D (~62) culture was observed and 2-5 fold decrease of CD29, CD73 and CD90 markers expression.
Our results showed that cytokine profiles secreted from MSC depend on the culture conditions and the source of the cells. ASC and WJ-MSC in 3D showed different secretion patterns of factors, which could be considered in different immunotherapy applications.

Acknowledgments: This work was supported by the National Science Fund, DCOST01/18.

P19

Dynamics of nuclear volume of spheroid cultured adipose tissue derived mesenchymal stroma cells

Radko Sotirov, Milena Kostadinova, Shina Pashova, Snejana Kestendjieva, Kameliya Vinketova, Desislava Abadjieva, Elena Stoyanova, Tsvetelina Oreshkova, Elena Kistanova, Milena Mourdjeva

Institute of Biology and Immunology of Reproduction, BAS

Abstract. The conventional method of cell cultivation in plasticware leads to the formation of a monolayer of cells with changed morphology and intercellular communications. Here, the method of the hanging drop is used as a technique for 3D cultivation of adipose tissue-derived mesenchymal stem cells (ASC) to study the morphological changes of cells, which compose the spheroids.

At day 3 and day 5 the size of ASC nuclei in the periphery and the center of spheroids were compared. The results showed a significant decrease of the nuclei dimensions (x; y), which changed from ~12-13 µm in 2D to ~6-8µm in 3D. Also taking into account the z dimention, the volumes of nuclei showed even more significant decrease: from 310-360 µm3 in 2D to 134 µm3 in the center and 109 µm3 in the periphery of 3D spheroids on day 3. On day 5, the nuclei doubled their volumes in 3D cultures becoming 273 µm3 in the center and 197 µm3 in the periphery of the spheroids. Similar results were also observed for the cells size. The nuclei of the cells in the center are larger than those in the edges of the spheroids. Additionally, the shape of the nuclei changed and

the cells in the spheroids showed sphere-like shape of nuclei, while the cells in the monolayer had ellipsoid-like nuclei.
In mesenchymal stem cells (MSC) biology cellular morphology is a key characteristic used to determine cellular phenotypes, ability for differentiation and fates of MSC. The necessity of adding a third dimention to the cell culture system, more closely resembles the natural environment and might generate significant differences in MSCs phenotype, behavior and therapeutic potential, which is increasingly recognized in the literature.

Acknowledgments: This work was supported by the National Science Fund, DCOST01/18.

P20

Isolation and characterization of human cremaster stem cells

Neia Naldaiz-Gastesi[1,2], María Goicoechea[1,2], Isabel Aragón[3], Bernardo Herrera-Imbroda[3], Adolfo Lopez de Munain[1,2,4,5], Maria Fernanda Lara-Cabanás[3,*] and Ander Izeta[6,*]

[1] Neuroscience Area, Biodonostia Institute, San Sebastian, Spain
[2] CIBERNED, Instituto de Salud Carlos III, Madrid, Spain
[3] Department of Urology, Virgen de la Victoria University Hospital, Malaga, Spain
[4] Faculty of Medicine and Dentistry, UPV-EHU, San Sebastian, Spain
[5] Department of Neurology, Hospital Universitario Donostia, San Sebastian, Spain
[6] Tissue Engineering Laboratory, Bioengineering Area, Biodonostia Institute, San Sebastian, Spain
**Corresponding authors, Ander Izeta <ander.izeta@biodonostia.org>; Maria Fernanda Lara Cabanás <fer76lc@hotmail.com>*

Abstract. Stress urinary incontinence (SUI) is a prevalent pathology for which available therapies present relatively frequent complications and do not target the underlying deficiency of the urinary sphincter. It represents an ideal target for cell-based therapies, due to the small volume of striated muscle tissue to be regenerated and its accessibility through minimally invasive surgery. Cell-based therapies of SUI currently being tested in clinical trials are based on MSCs or myoblasts. Both cell types are safe but, thus far, do not

seem to show sufficient efficacy. In this work, we aimed to isolate and characterize a muscle stem cell population from human cremaster muscle biopsies, and explore its clinical utility in the myogenic regeneration and treatment of male subjects suffering of urinary incontinence secondary to pelvic surgery. After in vitro expansion and differentiation, a robust myogenic differentiation of human cremaster muscle stem cells to striated myotubes was observed. The in vivo regenerative capacity was tested in a cardiotoxin-induced acute injury model performed on immunocompromised NSG mice. Human cremaster stem cells survived ectopic transplantation, generated new regenerative fibers, acquired Pax7 expression and colonized satellite stem cell niches in the damaged TA muscles. On the other hand, a murine SUI model based on neurogenic and muscular damage has been developed. This model faithfully represents the damage secondary to pelvic surgery and thus we are now ideally positioned to provide proof-of-concept studies of human cremaster muscle stem cells as a treatment of post-surgery SUI.

P21

Improving stemness and functional features of mesenchymal stem cells from Wharton's jelly of human umbilical cord by mimicking the native, low oxygen stem cell niche

Hristina Obradović, Jelena Krstić, Drenka Trivanović, Slavko Mojsilović, Juan F. Santibenez, Ivana Okić Đorđević, Tamara Kukolj, Vesna Ilić, Aleksandra Jauković, Diana Bugarski

Institute for Medical Research, University of Belgrade

Abstract. Mesenchymal stem cells (MSCs) from Wharton's Jelly of human umbilical cord (WJ-MSCs) gained attention as a potential tool in regenerative medicine based on their availability, proliferative potential and differentiation capacity. Since the physiological niche comprising WJ-MSCs is characterized by low oxygen levels ranging between 2% and 3%, we investigated whether cultivation of these cells at different oxygen levels affects their main functional features. Results showed that cultivation of WJ-MSCs under hypoxic conditions at 3% O2 did not influence their immunophenotype, while it modulated their differentiation process, and enhanced their clonogenic and expansion capacity when compared to cells grown at standard 21% O2. Besides that, 3% O2 induced transient change in cell cycle after 24h, and prevented cell death. The expression of pluripotency-associated marker genes NANOG, OCT4A, OCT4B and SOX2 was increased when cells were grown at 3% O2. Moreover, both cultivation and preculturing of WJMSC at 3% O2 increased their in vitro migration and mobilization

from collagen gel and enhanced the activity of extracellular matrix proteolytic enzymes uPA and MMP-2. We also observed that ERK1/2 mediates WJ-MSCs' mobilization from collagen drops regardless to oxygen levels, while Wnt/β-catenin pathway was activated during migration and mobilization at standard conditions. These data propose that culturing of WJ-MSCs under their microenvironment oxygen levels provide more representative results which reproduce the real situation in native niche and therefore should be considered as more credible conditions when investigating their stemness and functional properties for their potential implications in regenerative medicine.

P22

A combination of tissue engineering and regenerative medicine for two step whole-ovary in vitro reconstruction

Georgia Pennarossa[1], Matteo Ghiringhelli[1], Nicole Verdile[1], Fulvio Gandolfi[2] and Tiziana AL Brevini[1]

[1]Center for Stem Cell Research, Department of Health, Animal Science and Food Safety, University of Milan, Italy
[2]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy , University of Milan, Italy

Abstract. Ovarian failure is the most common cause of infertility and affects about 1% of young women. A possible therapy could be the combination of tissue engineering and regenerative medicine to achieve functional organ replacement. This strategy requires two main steps: A) whole-organ decellularization techniques that provides scaffolds consisting of naturally-derived extracellular matrix, with the maintenance of biological signals and tissue microarchitecture; B) efficient isolation of purified female germ stem cells (FGSC). Here we describe the development of the tools required for assembling ex vivo functional ovaries, using pig as a biomedical model.
Porcine ovaries were subjected to: A) whole-organ decellularization (triton X-100, sodium dodecyl sulfate and sodium deoxycholate for 36 hours) or B) FGSC isolation with different protocols: fragment culture; double enzymatic digestion; double enzymatic digestion followed by filtration or magnetic-activated cell sorting (MACS). Isolated cells were

grown up to 10 days, when cultures were arrested, and molecular analysis were performed.
Efficient cell removal from ovarian tissue was assessed by DNA quantification analysis, hematoxylin and eosin, and Masson's trichrome staining. Preservation of extracellular matrix structures was confirmed by histological studies. A highly enriched SSEA4+ FGSC population was obtained with MACS. These cells maintained significantly higher expression levels of pluripotency-related and germ cells specific genes, compared to those isolated with other tested techniques and, therefore, represent the best method for isolating functional cells for scaffold re-population.
Altogether, these results demonstrate the possibility to finalize both step A and B, providing two promising tools for whole-ovary bioengineering and reconstruction. This is a novel 3D platform for in vitro study of ovarian function.

Supported by Fondazione Carraresi and PSR2017

P23

Imaging intercellular interactions through different subpopulations of EVs

Sanchez-Lopez, Javier Arturo; Shuchmacher, Rita; Heifetz, Yael

Department of Entomology, The Hebrew University

Abstract. The use of extracellular vesicles (EVs) as mediators of cell communication, allows cells to transfer protected messages to highly specific target cells in short or long distances. The content of these messages are commonly bioactive molecules such as proteins or nucleic acids that can impact the behavior of the receiving cell. A variety of EVs like exosomes or microvesicles have been characterized by size, mechanism of secretion and the type of membrane protein "markers" that are embedded in the EVs membrane. In this way the characterization of EVs has become a useful method to reflect the homeostasis of the organisms.
We study the human uterine endometrial epithelial cells (EEC) and model intercellular communication through EVs. The EEC are highly plastic cells that line the uterine wall and interact directly with the developing embryo. Every menstrual cycle the EEC are functionalized by steroid hormones to shift their profile of expression to allow the implantation of an embryo. Coordination of the molecular interactions between EEC and the embryo is required for implantation success, and EVs might be essential mediators of the interactions. In this study, we developed in vitro EEC reporter lines that carry fluorescent proteins for a variety of membrane markers of EVs. Our goal is to understand the

dynamics of EEC communication through different subsets of EVs that could regulate different stages of cellular interactions for implantation.

P24

PLA composites and pure PLA decorated with cell-derived extracellular matrix impact on the fate of dental pulp stem cells osteogenic differentiation

Egidijus Simoliunas[1], Milda Alksne[1], Migle Kalvaityte[1], Virginija Bukelskiene[1], Daiva Baltriukiene[1]

[1]*Institute of Biochemistry, Life Sciences Center, Vilnius University, Sauletekio Ave. 7, LT-10223 Vilnius, Lithuania*

Abstract. Tissue engineering is aiming to develop biological substitutes to restore, replace or regenerate damaged or diseased tissues. In this field, the main goal of research is to combine stem cells from the body with optimal scaffold material composition and/or surface decoration. Such successful coincidence should promote cells to proliferate and at the right time to direct them to differentiate into a specific lineage. The aim of this work was to evaluate the impact of polylactic acid (PLA) scaffold composition and surface modifications on rat's dental pulp stem cells (DPSC) fate in vitro.

Porous 3D printed PLA composites with hydroxyapatite (10 %) or bioglass (10%) and cell-derived extracellular matrix (ECM)-coated PLA scaffolds were used to analyse their role in DPSC adhesion, migration, proliferation and osteogenic differentiation.

Obtained results indicated, that the best cell adhesion and proliferation were observed of DPSC which were grown on ECM-coated PLA scaffolds compared to composite ones.

However, the highest cell migration was detected on composite PLA-bioglass surface. Moreover, it was determined that the greatest osteogenic differentiation potential was registered in DPSC grown on ECM-coated PLA scaffolds. Overall, ECM proteins improved cell adhesion, proliferation and osteogenic differentiation compared to pure and composite PLA materials.

P25

Gal-3 regulates Kynurenine-dependent cross-talk between dendritic cells and T regulatory cells in ulcerative colitis

Simovic Markovic B[1], Gazdic Jankovic M[2], Acovic A[3], Arsenijevic N[1], Lukic ML[1] and Volarevic V[1]

[1]Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac
[2]Faculty of Medical Sciences, University of Kragujevac, Department of genetics
[3]Department for Dentistry, Faculty of Medical Sciences, University of Kragujevac

Abstract. Background. Galectin-3 (Gal-3) has an important role in inflammatory diseases, but its role in pathogenesis of chronic ulcerative disease (UC) is still incompletely defined. Dendritic cell (DC)-derived kynurenine (KYN) promote expansion of T regulatory cells in the colon of UC patients resulting in mucosal healing. Since Gal-3 is expressed on DCs and Tregs, we analyzed its importance for DC:Treg cross-talk in the gut.
Materials and method. We compared clinical and histological scores of dextran sodium sulphate-induced colitis in mice (DSS, 2.2%, molecular weight 40kDa) between Gal-3-deficient (Gal-3-/-) and wild-type (WT) C57BL/6 mice, and we analyzed expression of Gal-3 in serum samples of UC patients with mucosal healing and progressive disease and correlate it with serum levels of immunosuppressive KYN, as well as with the presence of T regulatory cells in lamina propria of UC patients.

Results. Gal-3 deficiency resulted in aggravation of DSS-induced colitis, indicating protective role of Gal-3 in this disease. Serum levels of KYN, as well as total number of lamina propria-infiltrated, immunosuppressive Tregulatory cells were attenuated in DSS-treated Gal-3-/- animals. TLR-2 activated and DSS-stimulated Gal-3-/- DCs produced lower amount of KYN compared with similarly treated WT DCs. In line with these results, serum concentration of Gal-3 was increased in UC patients with mucosal healing and decreased in UC patients with progressive disease. Serum levels of Gal-3 correlated with serum levels of KYN and with the total number of lamina propria-infiltrated Tregs

Conclusion. Gal-3 regulates Kynurenine-dependent cross-talk between dendritic cells and T regulatory cells in ulcerative colitis.

Keywords. Gal-3; ulcerative colitis; Kynurenine, dendritic cell, T regulatory cells

P26

Lineage fate of the human adipose tissue stem/stromal cells instructed by the extracellular matrix

Drenka Trivanović[1], Ivana Drvenica[2], Tamara Kukolj[1], Hristina Obradović[1], Ivana Okić Djordjević[1], Slavko Mojsilović[1], Jelena Krstić[1], Branko Bugarski[3], Aleksandra Jauković[1], Diana Bugarski[1]

[1] Laboratory for Experimental Hematology and Stem Cells, Institute for Medical Research, University of Belgrade, Serbia
[2] Laboratory for Immunology, Institute for Medical Research, University of Belgrade, Serbia
[3] Department of Chemical Engineering, Faculty of Technology and Metallurgy, University of Belgrade, Serbia

Abstract. An important constituent of the adipose tissue niche, extracellular matrix (ECM), provides physical and chemical support to mature adipocytes, immune cells and resident progenitor populations, referred as adipose tissue stem/stromal cells (ASCs). As the influence of ECM on ASC functions remains unclarified, the aim of our study was to reveal how ECM affects ASC differentiation. To delineate the intrinsic properties of ASCs and their response to external stimuli, we generated adipose tissue ECM-based biomimetic microenvironment that more accurately recapitulates conditions of native adipose stem cell niche.
We investigated the characteristics of fresh decellularized and delipidized ECM obtained from human adipose tissue samples, estimating its' appearance, mechanical features, protein content and cytocompatibility, by testing in vitro

adhesion and viability of ASCs and breast tumor cell lines. Besides, solubilized ECM-coating was applied to analyze ASC shape, metabolic activity, cytoskeleton changes and differentiation-associated events.

Nonlinear stress-strain behavior observed in tensile testing of fresh unsolubilized ECM and its' initial modulus were comparable to the values obtained for native subcutaneous adipose tissue. The fresh unsolubilized ECM provided a viable environment for both ASCs and tumor cells, suggesting that ECM retained original tissue features. In addition, 7-days cultivation of ASCs on solubilized ECM-coated surface, indicated that ECM regulates cytoskeleton organization, stimulates lipid droplet accumulation, and influences lineage commitment gene expression in ASCs. Solubilized ECM displayed the potential to upregulate mitochondrial membrane potential and Sirtuin1 expression, indicating important role of ECM as metabolic regulator in early phase of ASC adipogenesis.

Observed data suggest that both forms of ECM may retain tissue specific niche features, thus improving in vitro investigations of ASCs differentiation pathways at multiple maturation stages. Further efforts will support the deconvolution of the complex cell-ECM interactions which underlie adipose tissue homeostasis and diseases development, opening reasonable perspectives for the fabrication of ECM-based scaffolds and materials for the regenerative strategies.

P27

Characterization of the Intestinal Stem Cell niche in the rainbow trout

Nicole Verdile[1], Sharon Arcuri[2] , Tiziana A.L. Brevini[2], Fulvio Gandolfi[1]

[1]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, University of Milan, Italy
[2]Center for Stem Cell Research, Department of Health, Animal Science and Food Safety, University of Milan, Italy

Abstract. The intestinal tract complexity makes it difficult to predict the efficacy of different substances, or their combinations, without extensive in vivo trials. Restrictive rules and very high costs limit their use and, consequently, the number of substances that can be tested for efficacy and safety. The development of in vitro tests, able to screen compounds selected on theoretical considerations would be highly desirable. To this purpose, a better knowledge of the inner epithelial lining of the intestine is required, and the development of sophisticated 3-D culture systems that closely mimic the intestinal mucosa mandatory. Recently, the identification of robust adult intestinal stem cell markers in mouse and in humans, enabled the understanding of how intestinal stem cells interact with their local niche to maintain homeostasis in healthy intestinal epithelia. In domestic species including fishes the amount of available information is limited. In the present study, we describe our preliminary results on the characterization of the intestinal mucosa stem cell niche of five juvenile rainbow trout whose weight ranged between 44 and 61g. PCR analysis showed the

expression of leucine-rich-repeat-containing G-protein-coupled receptor 5 (LGR5) the typical marker of mammalian intestinal stem cells. We also detected the expression of SRY-box 9 (SOX9) that characterizes the transient amplifying population in mammalian intestinal crypts. Both molecules are known to be Wnt target genes, a key regulator of intestinal stem cell proliferation. Since intestinal crypts are not present in fish intestine we used an antibody specific for the Proliferative Cell Nuclear Antigen (PCNA) to localize proliferating cells. Our results showed a strong signal at the basis of the villi, confirming the stem/progenitor cell zone, is located within the intestinal folds in rainbow trout.

Supported by Skretting, University of Milan Transition Grant and EU H2020 Fish-AI grant n. 828835

P28

Reduced Apoptosis in Chinese Hamster Ovary Cells via Optimized CRISPR Interference

Kai Xiong[1], Kim Fabiano Marquart[1,2], Karen Julie la Cour Karottki[1*], Shangzhong Li[3,4,10], Isaac Shamie[3,10], Jae Seong Lee[5], Signe Gerling[1,6], Nan Cher Yeo[7], Alejandro Chavez[8], Gyun Min Lee[1,9], Nathan E. Lewis[3,4,10,§], Helene Faustrup Kildegaard[1,11,§]

[1]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Denmark.
[2]Integrated Biologics Profiling/Cell line development, Novartis, Basel, Switzerland (current address).
[3]Department of Pediatrics, University of California, San Diego.
[4]Department of Bioengineering, University of California, San Diego.
[5]Department of Molecular Science and Technology, Ajou University, Suwon, Republic of Korea.
[6]Novo Nordisk A/S, Hillerød, Denmark
[7]Department of Pharmacology and Toxicology, Precision Medicine Institute, University of Alabama, Birmingham, USA.
[8]Department of Pathology and Cell Biology, Columbia University College of Physicians and Surgeons, New York, New York, USA.
[9]Department of Biological Sciences, KAIST, Daejeon, Republic of Korea.
[10]The Novo Nordisk Foundation Center for Biosustainability, University of California, San Diego, United States.
[11]Novo Nordisk A/S, Måløv, Denmark (current address).

Abstract. Chinese hamster ovary (CHO) cells are widely used for biopharmaceutical protein production. One challenge limiting CHO cell productivity is apoptosis stemming from cellular stress during protein production. Here we applied CRISPR interference (CRISPRi) to downregulate the endogenous expression of apoptotic genes Bak, Bax, and Casp3 in CHO cells. In addition to reduced apoptosis, mitochondrial membrane integrity was improved and the caspase activity was reduced. Moreover, we optimized the CRISPRi system to enhance the gene repression efficiency in CHO cells by testing different repressor fusion types. An improved Cas9 repressor has been identified by applying C-terminal fusion of a bipartite repressor domain, KRAB–MeCP2, to nuclease-deficient Cas9. These results collectively demonstrate that CHO cells can be rescued from cell apoptosis by targeted gene repression using the CRISPRi system.

P29

Bone cells interaction at microfluidic platforms: establishment of tri-culture models to mimic bone innervation and vascularization

Neto E[1,2], Simões M[1,2], Leitão L[1,2,3], Sousa DM[1,2], Alves CJ[1,2], Conceição F[1,2,3], Chu V[4], Conde JP[4] and Lamghari M[1,2,3]

[1]i3S - Instituto de Investigção e Inovação em Saúde, Porto, Portugal
[2]INEB – Instituto de Engenharia Biomédica, Porto, Portugal
[3]ICBAS – Instituto de Ciências Biomédicas Abel Salazer, Porto, Portugal
[4]INESC MN - Instituto de Engenharia de Sistemas e Computadores - Microsistemas e Nanotecnologias, Lisboa, Portugal

Abstract. Bone homeostasis is a result of concerted interactions between bone cells and their surrounding microenvironment. Vascularization and innervation have been implied as fundamental players in bone development, homeostasis, and regeneration, and are often coupled both anatomically and physiologically. Current *in vitro* models lack the complexity required to study these crosstalks, since bone vascularized models do not usually include innervation and *vice-versa*.
Herein, a triculture composed of sensory neurons, endothelial cells, and osteoclasts was assembled within a microfluidic device, given the possibility of culturing different cell types under precisely controlled conditions.

The optimization of the 3D culture of human umbilical vein endothelial cells within different hydrogels with angiogenic potential: Matrigel®, fibrin and collagen/fibrin mixture was conducted. After 72 hours, cells were found to coat the microchannels forming a lumen-like structure. Axonal outgrowth occurred from the neuronal compartment towards the endothelial cells, possibly indicating a supportive effect of the endothelial cells on the nerve growth.

In this work we provide a sophisticated and complex tri-culture *in vitro* model, mimicking the vascularized and innervated bone tissue, aiming to reduce or even replace the *in vivo* assays.

Participants

Name	Email Adress	Country
Agnieszka Waclawik	waclawik@pan.olsztyn.pl	Poland
Aleksandar Acovic	dr.acovic115@gmail.com	Serbia
Aleksandar Arsenijevic	aleksandar@medf.kg.ac.rs	Serbia
Aleksandra Jauković	aleksandra@imi.bg.ac.rs	Serbia
Aleksandra Uskoković	auskokovic@ibiss.bg.ac.rs	Serbia
Algirdas Ziogas	algirdas.ziogas@imcentras.lt	Lithuania
Amos Tandler	tandleramos@gmail.com	Israel
Ana Sarić	anasaric29@gmail.com	Serbia
Andras Dinnyes	Andras.dinnyes@biotalentum.hu	Hungary
Andreia Ferreira de Castro Gomes	agomes@bio.uminho.pt	Portugal
Aneta Andronowska	a.andronowska@pan.olsztyn.pl	Poland
Anja Tolić	anja.tolic@ibiss.bg.ac.rs	Serbia
Anne Navarrete Santos	a.navarrete-santos@medizin.uni-halle.de	Germany
Ayse Eser ELCIN	aeserelcin@gmail.com	Turkey
Barbara Azzimonti	barbara.azzimonti@med.uniupo.it	Italy
Bart M Gadella	b.m.gadella@uu.nl	Netherland
Bartosz Doktór	bartosz.doktor@realresearch.life	Poland
Bernard AJ Roelen	b.a.j.roelen@uu.nl	Netherland
Bojana Djokovic	bojanadjokovic86@yahoo.com	Serbia
Bojana Simovic Markovic	bojana.simovic@gmail.com	Serbia
Bojana Žegura	bojana.zegura@nib.si	Slovenia
Daiva Baltriukienė	daiva.baltriukiene@bchi.vu.lt	Lithuania
Dawit Tesfaye	tesfaye@itw.uni-bonn.de	Germany
Denis Khnykin	denis.khnykin@rr-research.no	Norway

Diana Bugarski	dianab@imi.bg.ac.rs	Serbia
Drenka Trivanović	drenka.trivanovic@gmail.com	Serbia
Egidijus Simoliunas	Egidijus.simoliunas@gmc.vu.lt	Lithuania
Elena Kistanova	kistanova@gmail.com	Bulgaria
Estrela Neto	Estrela.netoi@ineb.up.pt	Portugal
Gabriel Furtos	gfurtos@yahoo.co.uk	Romania
Georgia Pennarossa	Georgia.pennarossa@unimi.it	Italy
Giovanna Brusatin	giovanna.brusatin@unipd.it	Italy
Goran Poznanovic	goranpoz@ibiss.bg.ac.rs	Serbia
Grigorova, Natalia Tsoneva	n.gr@abv.bg	Bulgaria
Hristina Obradovic	hristina.obradovic@imi.bg.ac.rs	Serbia
Ioana-Codruta Mirica	mirica_codruta@yahoo.com	Romania
Isabel Freitas Amaral	iamaral@ineb.up.pt	Portugal
Jennifer Schön	schoen.jennifer@fbn-dummerstorf.de	Germany
Joana Saraiva Rodrigues	Joana.s.rodrigues@campus.ul.pt	Portugal
Joao Teixeira	Jpft12@gmail.com	Portugal
Jolanda VAN HENGEL	Jolanda.vanhengel@ugent.be	Belgium
Jovana Rajić	jovana.rajic@ibiss.bg.ac.rs	Serbia
Juliane-Susanne Jung	juliane-susanne.jung@uk-halle.de	Germany
Klara Hercog	klara.hercog@nib.si	Slovenia
Maja Miletić	maja.miletic@stomf.bg.ac.rs	Serbia
Maja Misirkic Marjanovic	mmisirkic@yahoo.com	Serbia
Marcin Krzykawski	marcin.krzykawski@realresearch.life	Poland
María Fernanda Lara Cabanás	Fer76lc@hotmail.com	Spain
Marija Sinadinović	marija.sinadinovic@ibiss.bg.ac.rs	Serbia
Marina Gazdic Jankovic	marinagazdic87@gmail.com	Serbia

Marta Muñoz	mmunoz@serida.org	Spain
Martina Štampar	Martina.stampar@nib.si	Slovenia
Melita Vidakovic	melita@ibiss.bg.ac.rs	Serbia
Meriem Lamghari	lamghari@ineb.up.pt	Portugal
Michal Ješeta	jeseta@gmail.com	Czech Republic
Milda Alksne	milda.peciukaityte@gf.vu.lt	Lithuania
Milena Sergeeva Mourdjeva	milena_mourdjeva@abv.bg	Bulgaria
Miodrag Lukic	miodrag.lukic@medf.kg.ac.rs	Serbia
Mirjana Mihailovic	mista@ibiss.bg.ac.rs	Serbia
Murat Elcin	elcinmurat@gmail.com	Turkey
Nevena Grdović	nevenag@ibiss.bg.ac.rs	Serbia
Nicole Verdile	Nicole.verdile@unimi.it	Italy
Ortega Nicolas Matias	Nicolas.ortega@ki.se	Sweden
Peter Ertl	peter.ertl@tuwien.ac.at	Austria
Radostina Alexandrova	rialexandrova@hotmail.com	Bulgaria
Samuel Gebremedhn	seta@itw.uni-bonn.de	Germany
Sanja Momcilovic	sanja.momcilovic@imi.bg.ac.rs	Serbia
Sanja Vignjević Petrinović	sanja.vignjevic@imi.bg.ac.rs	Serbia
Sérgio José Póvoas Camões	sergiocamoes@campus.ul.pt	Portugal
Sergio Ledda	giodi@uniss.it	Italy
Sharon Arcuri	sharon.arcuri@unimi.it	Italy
Sharon Schlesinger	sharon.shle@mail.huji.ac.il	Israel
Shuai Chen	chen@fbn-dummerstorf.de	Germany
Slavko Mojsilović	slavko@imi.bg.ac.rs	Serbia
Stefania Pizzimenti	stefania.pizzimenti@unitio.it	Italy
Svetlana Dinić	sdinic@ibiss.bg.ac.rs	Serbia

Tamara Kukolj	kukoljtamara@gmail.com	Serbia
Tiziana Angela Luisa Brevini	tiziana.brevini@unimi.it	Italy
Vachkova, Ekaterina Georgieva	katvach@gbg.bg	Bulgaria
Vladimir Trajkovic	vtrajkovic@med.bg.ac.rs	Serbia
Vladislav Volarevic	drvolarevic@yahoo.com	Serbia

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

602.9(048)

CELLFIT Workshop (2 ; 2019 ; Beograd)
Cells Communicate! : Book of Abstracts of the Second CellFit

Workshop 2019 Belgrade, Serbia 13 - 14 March, 2019. /
editors Tiziana Brevini ...[et al.]. - Belgrade : Institute for
Biological Research "Siniša Stanković", University ; Brussels :
COST Action CA16119, 2019 (Belgrade : Copy Planet).

(Belgrade : Copy Planet). - 104 str. ; 21 cm

Tiraž 70.

ISBN 978-86-80335-08-7 (IBRSSU)

a) Матичне ћелије - Апстракти

COBISS.SR-ID 274380812