IN VITRO 3-D TOTAL CELL GUIDANCE AND FITNESS

PROCEEDINGS OF CellFit MEETING 2018

2nd-3rd of October 2018

Hvar Island
Croatia

Editors

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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

Publisher: School of Medicine University of Zagreb

Book Title: In Vitro 3D Total Cell Guidance and Fitness

Year of Publication: 2018

ISBN: 978-953-6255-70-2

978-953-6255-69-6

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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.

COST 16119 CellFit, Annual Meeting Hvar, Croatia, 2-3 October 2018

Understand how cells react to stimuli deriving from cell-to-cell communications as well as from mechanical cues, such as tension, compression and fluid flow. Elucidate the multitude of cell membrane receptors, and their coupling to the cytoskeleton to change cell shape and functional state in response to cell-cell contacts. Analyse the pathways activated by modifications of cell adherence properties, induced by the surrounding environment, and local mechanical stimuli.

Welcome address From the Action Chairman

Welcome to the second Annual Meeting of our COST Action CA16119, that will take place in Hvar, a pearl of the Adriatic, an island of stunning beauty, nominated for best destination 2018.

We will enjoy great scientific days, with outstanding speakers from many different COST countries, and will discuss how cells react to stimuli deriving from cell-to-cell communications as well as from mechanical cues. We will analyse the pathways activated by modifications of cell adherence properties, induced by the surrounding environment, and local mechanical stimuli

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

I am really grateful to Ana Katušic 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 and Georgia May for

managing the technical aspects.

And thanks to all of you, for your enthusiastic help and support,

Dobro došli

Prof Tiziana A.L. Brevini

Chair of CellFit

October 2018

PROGRAMME

Day 1, Monday, 1st October 2018

Arrival and accommodation

19.00-21.30 Dinner – at Hotel Pharos

Day 2, Tuesday, 2nd October 2018

08.00-09.00 Registration

09.00-09.15 Welcome Address (Local MC Members and Action Chair)

Working Group 1:

09.15-09.45 *Blastoid: modelling the preimplantation embryo (O-43)* **Frias-Aldeguer, Javier**

09.45-10.15 *3D tumorspheres as models to assess cellular responses to chemotherapy (O-45)* **Walsh, Naomi**

10.15-10.30 *Printable Composite Bioink for Periodontal Tissue Engineering (O-10)* **Elcin, Yasar Murat**

10.30-11.30 Coffee break and Poster session (odd number)

11.30-11.45 *Simple method of creating microfluidic structures using 3D printing (O-39)* **Karcz Adriana**

11.45-12.00 *Characterization of three dimensional (3D) neurospheres for developmental toxicology studies (O-9)* **Dinnyés, András**

12.00-12.15 *Bioengineering the Structure of the Bovine Endometrium Using Advanced Cell Culture technology (O-19)* **Muñoz, Marta**

13.00-14.00 **Lunch Break – at Hotel Amfora**

14.00-16.00 **Networking and informal discussion**

Working Group 2:

16.00-16.30 *Circularization of genes and chromosomes by CRISPR in human cells (O-46)* **Luo, Yonglun**

16.35-17.10 *Scaffold micropatterning for peripheral nerve regeneration (O-47)* **Sannino, Alessandro**

17.15-17.30 *Mechanical cues boost pancreatic differentiation via the YAP/TAZ signaling pathway (O-7),* **And,** *Development of a methacrylated gelatin-based 3D*

Artificial Intestine platform (O-20)

<u>Brevini, Tiziana Angela Luisa, And Verdile, Nicole</u>

17.30-17.45 *Post-translational α-tubulin modification in 3D spheroids of rat glioma cells developed in transparent liquid marbles and hanging drops (O-2), And, Whole decellularized pig heart for cardiac tissue engineering: a new low flow/pressure method (O-11)*

<u>Ledda, Sergio And Ghiringhelli, Matteo</u>

17.45-18.00 *Engineered substrates with site-specific immobilized laminin for modulation of neural stem cell behaviour (O-6)* **<u>Amaral, Isabel Freitas</u>**

18.00-18.15 *Ruthenium(III) complexes containing Schiff base ligands suppress 2D and 3D growth of cancer cell (O-5)* **<u>Alexandrova, Radostina</u>**

19.00-21.30 **Dinner – at Hotel Pharos**

Day 3, Wednesday, 3rd October 2018

09.00-09.15 Presentation of COST rules reimbursement

Working Group 3:

09.15-09.45 ***Nanotopographies transmit physical information to the genome through modulation of the nuclear lamina (O-48)*** **Sero, Julia**

09.45-10.15 ***Generation of exosome heterogeneity through conserved endosomal trafficking routes: from flies to human cancer cells (O-40)*** **Wilson, Clive**

10.15-10.30 ***Deciphering embryo-maternal communication; quantification of the dynamics of trophoblastic extracellular vesicle mediated language (O-12)*** **Godakumara, Kasun**

10.30-11.30 Coffee Break and Poster session (even numbers)

11.30-11.45 ***Analysis of human neural stem cell secretome during neuronal differentiation in vitro (O-31)*** **Vodicka, Petr**

11.45-12.00 ***Dynamic landscape of peri-cenromeric heterochromatin during transition from naïve to primed pluripotency (O-15)*** **Jouneau, Alice**

12.00-12.15 ***Priming hnMSCs' secretome using 3D spheroid cultures for the treatment of cutaneous wounds (O-18)*** **Miranda, Joana Paiva**

13.00-14.00 **Lunch Break – at Hotel Amfora**

15.00-16.00 **MC meeting**

Working Group 4:

16.00-16.30 ***Evolution in 3D cell culture: synthetic hydrogel with unique biomechanical properties (O-34)*** **Juliette Van den Dolder**

16.35-17.05 ***Biofabrication, microfluidics, organs-on-chips and bioreactors (O-41)*** **Costa, Pedro**

17.10-17.25 ***Liquid pearls for anticancer drug discovery (O-35)*** **Muller, Christian, D.**

17.30-17.45 ***Mimicking the maternal environment to optimize the in vitro production of embryos in wild and***

domestic mammals (O-17) **Mermillod, Pascal**

17.45-18.00 *Mesenchymal stem cells in self-assembled spheroids (O-16)* **Kukolj, Tamara**

18.00-18.15 *3D tissue engineered cartilage grafts for the repair of patella femoral osteochondral kissing lesions (O-23)* **Pušić, Maja**

18.15-18.45 Conclusive remarks and General Discussion

20.30-01.00 Gala Dinner

Day 4, Thursday, 4th October 2018

09.00-15.30 Excursion to Paklinski Islands Boat trip

ABSTRACTS OF PRESENTATIONS

Oral Presentation (O-5)

Alexandrova, Radostina
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

Co-Authors:
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Ruthenium(III) complexes containing Schiff base ligands suppress 2D and 3D growth of cancer cells

The aim of our study was to evaluate the effect of three newly synthesized ruthenium(III) complexes with Schiff bases

resulted from the condensation reaction between salicylaldehyde and ethylenediamine (Salen), 1,3-diaminopropane (Salpn) and 1,2-phenylenediamine (Salphen), respectively on 2D and 3D growth of cancer cells. The following cell lines were used as model systems in our study: MDA-MB-231 (human triple negative breast cancer), HeLa (human cervical carcinoma), LSR-SF-SR (rat sarcoma) and Lep-3 (non-tumor embryonic fibroblastoid cells).

The investigations were performed in short-term experiments (3h-72h, with monolayer cultures) using MTT test, neutral red uptake cytotoxicity assay, crystal violet staining, double staining with acridine orange and propidium iodide; haematoxylin and eosin staining; AnnexinV/FITC method as well as in long-term experiments (up to 50 days) by 3D colony-forming method.

The obtained results reveal that applied at concentrations in the range 5-50 μg/ml the investigated complexes sigbificantly decreased 2D and 3D growth of cancer cell. Ru(III) complex with Salpn has been found to be the most promising cytotoxic agent among the compounds tested but is less effective as compared to commercially available antitumor agents cisplation, oxaliplatin and epirubicin.

This study was supported by COST Action CA16119 "Cellfit" and a bilateral project between Bulgarian Academy of Sciences and Romanian Academy.

Oral Presentation (O-6)

Amaral, Isabel Freitas
i3S - Instituto de Investigação e Inovação em Saúde; INEB - Instituto de Engenharia Biomédica, U. Porto, Porto, Portugal

Co-Authors:
Barros, D.; Parreira, P.; Furtado, J.; Ferreira-da-Silva, F.; García, A.J.; Martins, M.C. L.; Pêgo, A.P..

Engineered substrates with site-specific immobilized laminin for modulation of neural stem cell behaviour

Laminin (Ln) immobilization into extracellular matrix (ECM) analogs is being actively pursued to replicate the microenvironment of different stem cell niches. However, previous attempts to immobilize Ln have been limited to non-specific immobilization approaches, which may compromise the exposure of crucial Ln bioactive epitopes, as those interacting with cell surface receptors and with other ECM molecules.

Herein we established an affinity-based approach that explores the high affinity interaction between a recombinant human fragment of an ECM component (rhX) and the Ln triple α-helical coiled-coil, to control the immobilization of recombinant human Ln (rhLn-521) on both 2D model surfaces (SAMs of mono-PEGylated rhX on gold) and within protease-sensitive PEG-based hydrogels. We show that the site-specific immobilization of rhLn better preserves protein bioactivity in comparison to that immobilized on SAMs of thiol-PEG-

succinimidyl glutaramide (used for non-selective covalent immobilization of Ln), as evidenced by Ln enhanced ability to self-polymerize and mediate cell adhesion and spreading of human neural stem cells (hNSCs). Moreover, the protease-sensitive hydrogel containing affinity-bound Ln was shown to support hNSC viability and neuronal differentiation.

These results highlight the potential of this novel strategy to be used as an alternative to the conventional immobilization approaches in a wide range of applications, including engineered coatings for cell culture and biofunctionalization of 3D matrices.

Supported by projects NORTE-01-0145-FEDER-000008 and NORTE-01-0145-FEDER-000012, financed by NORTE 2020, FEDER funds through COMPETE 2020 – POCI, and National Funds through FCT/MCTES in the framework of the project "Institute for Research and Innovation in Health Sciences" (POCI-01-0145-FEDER-007274).

Oral Presentation (O-7)

Brevini, Tiziana Angela Luisa
Laboratory of Biomedical Embryology, Center for Stem Cell Research, Università degli Studi di Milano, Italy.

Co-Authors:
Manzoni, Elena Franca Maria; Arcuri, Sharon; Gandolfi, Fulvio; Brevini, Tiziana Angela Luisa

Mechanical cues boost pancreatic differentiation via the YAP/TAZ signaling pathway

Epigenetic modifiers directly interact with the signature of an adult mature cell, allowing to change its phenotype. In particular, the molecule 5-azacytidine (5-aza-CR), which is able to interfere with DNA methylation, through both a direct and an indirect effect, can be used to remove the epigenetic 'blocks' responsible for tissue specification and to facilitate cell transition to a different lineage. We have recently shown that this process is influenced by 3D rearrangements and mechanical properties of the cellular microenvironment. In the experiments here described, we investigated, in particular, the effect of a selected 3D culture system. We used INS-eGFP porcine fibroblasts, that express enhanced GFP (eGFP) under the control of insulin gene promoter, as experimental model, and wild-type pig fibroblasts, as control. Both cell types, were plated, either on plastic or on 1kPa polyacrylamide (PAA) gel, that mimics the stiffness of the pancreatic tissue in vivo. Epigenetic erasing was performed with 5-aza-CR. Cells were

then driven towards pancreatic differentiation using a dedicated protocol. The use of INS-eGFP fibroblasts allowed real-time monitoring of progression towards the pancreatic phenotype. Morphological analysis and pancreatic marker expression were checked for the entire length of the experiment.

PAA gels encouraged the induction of islet-like structures, confirming that the formation of 3D clusters is a crucial aspect of pancreatic differentiation in vitro. Moreover, the use of an adequate substrate accelerated differentiation and anticipated cell insulin secretion ability. A direct implication of YAP/TAZ mechanotransduction-mediated pathway was demonstrated, suggesting that mechanical cues have a crucial role in pancreatic phenotype definition.

Supported by Carraresi Foundation.

Poster Presentation (P-8)

Bunoza, Paula
Department of Medical Biology, School of medicine University of Zagreb, Croatia

Co-Authors:
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The temperature and culture conditions affect rat limb bud development in an ex vivo 3D organ culture model

The aim of our research was to create an ex vivo 3D organ culture model of the mammalian limb bud development for exploring the effect of temperature changes without the influence of the maternal organism. 13 and/or 14 days old limb buds were microsurgically isolated from Fisher rat embryos and cultured from 3 to 14 days in media supplemented with

serum or chemically defined media at normothermia (37 ºC), the two-week hypothermia (31 ºC) and hyperthermia (43 ºC, 15 min). The overall growth of explants was measured and standardized on the day of plating (A0). The numerical density (Nv) of the cells positive for marker of cell proliferation (PCNA) was assessed. The overall methylation was analysed by pyrosequencing to explore the differences in DNA methylation due to the cultivation conditions and developmental stage.
Ex vivo experiments revealed the progress of differentiation of 3D explants into cartilage, epidermis and myotubes. Overall growth, Nv of the PCNA positive cells and histomorphology were most affected in pure MEM.

On hyperthermia, significant negative impact on the overall growth in the three-day cultures with serum was discovered, but the density of PCNA positive cells was significantly higher in both the serum supplemented and MEM culture conditions. In the two-week hypothermia overall growth did not differ depending on the media, or of normothermia. However, PCNA expression was significantly lower than in the control with serum at normothermia. The DNA methylation seemed also to be differentially changed due to experimental conditions.
This original 3D ex vivo model had discovered specific influences of temperature changes and culture conditions on the limb development. That is of importance for a better understanding of possible side-effects of physical therapy on limb bud stem cells.

Guest speaker (O-40)

Clive, Wilson
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Generation of exosome heterogeneity through different stress-regulated endosomal trafficking routes

Secreted extracellular vesicles, including endosome-derived exosomes, can mediate multifaceted intercellular signalling events by means of their complex combinations of protein, nucleic acid and lipid cargos. In the 'classical' exosome pathway, intraluminal vesicles (ILVs) formed in late endosomal multivesicular bodies (MVBs) are released as exosomes when these compartments fuse to the plasma

membrane. We have investigated whether other subcellular compartments generate exosomes in normal and cancer cells, and whether this might partly explain the heterogeneity observed in exosome preparations.

To initially address this question, we developed an *in vivo* 3D organ preparation to observe and genetically manipulate the subcellular events of exosome biogenesis. We used a *Drosophila* prostate-like model in which exosomes are normally secreted from highly enlarged compartments.

We demonstrate through super-resolution 3D-structured illumination microscopy (3D-SIM) that exosomes carrying distinct cargos, including the small GTPase Rab11, are formed inside compartments marked by recycling endosomal Rab11.

We confirm that different subtypes of exosome are generated by late and recycling endosomes in several human cancer cell lines.

Depleting these cells of extracellular glutamine or inhibiting signalling by growth regulatory and nutrient-sensitive mechanistic Target of Rapamycin (mTORC1) can induce an 'exosome switch' in which the alternative Rab11-positive exosomes are preferentially released via altered endosomal flux. These exosomes have a distinct 'Rab signature' and altered signalling activity, preferentially maintaining endothelial networks and driving ERK-MAPK-dependent cancer cell growth. The switched exosomes contribute to adaptive responses in tumour cells. Further studies of the 3D fly model have revealed additional novel mechanisms of secretion that can now be pursued biochemically with 2D human cell models to ascertain whether they are evolutionarily conserved.

Guest speaker (O-41)

Costa, Pedro
BIOFABICS - 3D Biotissue Analogues, Vizela/Porto, Portugal

Biofabrication, microfluidics, organs-on-chips and bioreactors

It is becoming increasingly evident that standard tissue models, such as 2D *in vitro* cultures and *in vivo* animal models, may not be fully representative of native human tissues. Various industries, including pharma and cosmetics, are in need of improved and more reliable tissue models in order to generate products more efficiently. Many research groups and companies are developing such models independently and resorting to individual technologies. At BIOFABICS we believe that the ability to generate reliable tissue models as well as tissue substitutes must result from the combination of various technologies and know-how from multiple sources. Therefore, BIOFABICS draws its expertise and know-how from its international team's decades of research and innovation activities at the intersection of many fields such as biofabrication, microfluidics, organ-on-chip technologies, bioreactor technologies, tissue engineering & regenerative medicine, biomaterials, cell & tissue biology as well as mechatronics & software development. In this talk, it will be shown how the ability to collaborate and combine technologies and know-how across borders and sectors is able to more efficiently advance science and technology.

Oral Presentation (O-9)

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1. BioTalentum Ltd. H-2100, Gödöllő, Aulich L. utca 26. Hungary

2. Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest H-1117; Hungary

Characterization of three dimensional (3D) neurospheres for developmental toxicology studies

Three-dimensional cell cultures have shown more physiological relevance compared to traditional two-dimensional systems. Differentiation of patient-derived induced pluripotent stem cells (iPSCs) into 3D free-floating neurospheres provides advantages to study relevant neurological pathways. 3D spheres also allow preclinical analyses of selected neural drug candidates and could be a promising tool for neuro-toxicology.
Here we present the detailed characterisation of three-dimensional neurospheres differentiated from control iPSCs. Spheres were differentiated up to 7 weeks, samples were collected, fixed, cryosectioned and immunoassayed at weekly intervals. Moreover, samples were analysed with electron microscopy to follow ultrastructural changes and synaptic

maturity. The number of dividing cells were decreasing continuously (Ki67 positive cell number), while neuronal marker expression, such as TUBB3, MAP2, NF200 were increasing with maturity. Synaptic marker VAPM2 showed strong expression from week 5, while Vglut1/2, VaCHT and GAD65/67 expression was detectable from week 4.
Moreover, astrocytes differentiation (AQP4 and GFAP) and oligodendrocyte differentiation (MBP) was also investigated. Electron microscopy results supported the above findings. In conclusion, our results showed that the iPSC-based 3D spheroid system efficiently mimics the neuronal differentiation and suitable for developmental neurotoxicity studies.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002 and 'CellFit' COST Action Project (16119).

Oral Presentation (O-10)

Elcin, Yasar Murat
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Printable Composite Bioink for Periodontal Tissue Engineering

Periodontal diseases are a group of diseases with multi-agent etiology, with an indirect cost of ~ 442 billion dollars globally (WHO 2010). They are treated by non-invasive and invasive techniques; however, current therapies and preventive approaches are far from sufficient. Some candidate approaches from fields of regenerative medicine and tissue engineering has not yet succeeded in developing a sufficient cure or a novel drug molecule. This can be attributed to the significant difficulties in simulating the micro-environment of the periodontal tissue. Recently, 3D-bioprinting systems are used to develop microconstructs that have optimal micro-architecture and cell positioning by using biological molecules and cells with a carrier bioink in gel form. Although there are microfluidic chip studies involving the 3D-bioprinting of the cartilage, bone, skin and nerve tissue, our endeavour for modeling of the periodontal tissue in a similar setting is a new attempt. In this study, we have developed a hybrid composite bioink gel system that can mimic the natural periodontal tissue microenvironment including the inorganic components under

in vitro conditions. The developed composite bioink was characterized by using a number of methods, including FTIR, SEM, and by rheological analyses.
It was found that, the composite bioink had gelation kinetics suitable for 3D-bioprinting. Cell culture studies indicated that the 3D-bioprinted microtissues supported viability of the cells. Our final goal is to develop the periodontal tissue microenvironment for prospective in vitro drug/disease modeling studies.

This study is financially supported by TUBITAK (117M281).

Guest Speaker (O-43)

Frias Aldeguer, Javier
MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands.

Co-Authors:
Vrij, Erik J.; Li, Linfeng; Rivron, Nicolas

Blastoid: modelling the preimplantation embryo

Prior to implantation, the mammalian embryo undergoes the first lineage commitment, resulting in an embryonic and an extraembryonic compartment present at the blastocyst stage: the inner cell mass represents the embryonic tissue that will lead to the formation of the embryo proper; and the trophectoderm that will form the placenta. Embryonic stem cells (ESC) and trophoblast stem cells (TSC) are the *in vitro* analogs of those two compartments and have been available *in vitro* for decades, allowing us to study several processes taking place during embryonic development. Classical *in vitro* models have been a constant source of knowledge, but in most fields they fail to represent the environmental context observed *in vivo*. Recently more complex cultures have been developed by combining multiple cell types or by culturing in 3D. In the field of embryology, one of those new models combines both ESC and TSC in order to generate the blastoid[1], a 3D organoid that morphologically and functionally resembles the preimplantation embryo. Blastoids recapitulate key events in the biology of the peri-implantation embryo, such as cavitation, embryonic-extraembryonic crosstalk, implantation or decidualization. The protocol we have developed allows us to

consistently obtain a large number of Blastoids making it an attractive tool for performing large scale studies such as drug screenings or compartment specific genetic studies.

1. Rivron NC, Frias-aldeguer J, Vrij EJ, et al. Blastocyst-like structures generated solely from stem cells. 2018.

Oral Presentation (O-11)

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Co-Authors: Kazuya Mamada, Masami Uechi, Tiziana A.L. Brevini

Whole decellularized pig heart for cardiac tissue engineering: a new low flow/pressure method.

New approaches for organ transplantation have been developed in the last few years and the innovative field of organ engineering is rapidly evolving, to prepare and create new high-tech biological scaffolds. Whole organ engineering would benefit from three-dimensional scaffold produced from intact organ-specific extracellular matrix (ECM). To this purpose, customized decellularization protocols are mandatory. Aim of this study was 1) to create a whole decellularized heart, preserving the original organ ECM architecture. 2) to maintain unaltered organization of Mitral and Tricuspid valves, as well as left and right atrium. This was implemented using a medium/long perfusion time and a low flow/pressure circuit, applied to pig hearts, collected from the local slaughterhouse. Whole porcine hearts were treated for 30 hours with 1 % Sodium dodecyl sulfate (SDS), 2X Phosphate Buffered saline (PBS), 0,1% Ammonium hydroxide solution (NH_4OH), followed by quantitative and qualitative analysis. Hematoxylin and eosin staining confirmed the removal of cellular material. At the same time, immunohistochemistry demonstrated

preservation of the native extracellular matrix proteins, including heparan sulfate proteoglycan, fibronectin, collagen types I and IV, and laminin. Altogether, decellularization agents, incubation temperatures and pressure/flow values selected in the present study, demonstrated to be effective to preserve the organ native architecture.

Oral Presentation (O-12)

Godakumara, Kasun
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Co-Authors:
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Deciphering embryo-maternal communication; quantification of the dynamics of trophoblastic extracellular vesicle mediated language.

Communication between pre-implantation embryo and the endometrium plays a vital role in endometrium receptivity. Our laboratory has previously demonstrated that several transcripts are transferred from the trophoblast to the endometrial cells through extracellular vesicles (EVs) and induce transcriptomic changes in endometrial cells following the transfer. The objective of the current study was to investigate the involvement of EVs in the transfer of candidate transcripts from trophoblast to endometrium. Spheroids derived from JAr and RL 95-2 cell lines were used as analogues for trophoblast and endometrium cells, respectively. EVs from trophoblasts were isolated using size exclusion liquid chromatography. Endometrial cells were treated with purified EVs in series of increasing incubation time and/or EV amount. The change of gene expression in endometrial cells were measured using

qPCR with absolute quantification and were compared to untreated controls and controls treated with EVs derived from HEK (Human Embryonic Kidney) cell spheroids. Trophoblastic EVs induced transcriptomic changes in endometrial cells and the effect was specific to trophoblast spheroid derived EVs, required relatively low concentration of EVs per recipient cell (approx. 4) and resulted in relatively rapid response from the host cells (<60 min). These results help to lead the way in understanding the language of communication between the mother and baby during early stages of pregnancy.

Poster Presentation (P-13)

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Co-Authors:
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Advanced cell culture models for the study of traditional African phytomedicines

Traditional herbal medicine remains popular in Africa, with an estimated 80% use in some parts. In certain regions it is often the main or sometimes even the only healthcare service available, especially in the most rural areas. The African continent also has a rich biodiversity, contributing to accessibility to diverse medicinal plants. Although many benefits have been derived from the use of traditional herbal medicines, many concerns are associated with their use. This includes unknown transport characteristics, unknown toxicity levels and potential herb-drug interactions which have been identified to have a rising impact on patient treatment outcomes. It is clear from our studies that traditional African medicines can modify pharmacokinetics properties of co-administered drugs, while frequently used herbal medicines have proven to be toxic. However, literature to this regard is limited and often contradictory, highlighting the need for more research. To study these phytomedicines, physiologically relevant models are essential to reduce the discrepancies between in vitro data and case studies. This requires different

advanced cell culture models for each research question, and can range from dynamic three-dimensional spheroid cultures to air-liquid interface models.

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Poster Presentation (P-3)

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Prostaglandin secretion by the early bovine embryo during 24h of culture

The development of bovine embryos in vitro is limited due to unsuitable culture conditions. This limitation might be improved by the development of a more natural culture environment. To do so, it is essential to know the signaling factors produced by the maternal organism and the embryo that are involved in early embryonic development in vivo. Prostaglandins (PG) contribute to the cross-talk between the maternal organism and the embryo supporting early embryonic development. During the pre-implantation period a shift from prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to prostaglandin E_2 (PGE_2) production has been considered to be a prerequisite in cattle. However, own earlier findings revealed no difference in the intrauterine $PGF_{2\alpha}/PGE_2$ ratio when comparing pregnant and cyclic animals. Furthermore, 6-keto $PGF_{1\alpha}$ that is the stable metabolite of PGI_2 was found to be the predominant PG in the uterus of pregnant cattle. Therefore, other PG than PGE_2 might play an important, but to date unknown role during early pregnancy. For that reason, we established an UP-LC-MS platform that is able to detect 33 different PG, including not

only 2-series PG and their metabolites but also 1-series and 3-series PG and their metabolites. PG secreted by in-vivo produced embryos at different developmental stages (days 6, 8, 10, 12 and 14, spanning the developmental states unhatched blastocysts until the start of trophoblast elongation) are currently under analysis. Preliminary measurements of samples (day 12 and day 14 embryos) revealed that $PGF_{2\alpha}$ is probably the predominant PG produced by the early bovine embryo.
PGE_2 and $PGF_{1\alpha}$ are also produced in considerable amounts. The preliminary data evidence that multiple PG are synthesized by bovine embryos and may be of considerable relevance during early embryonic development. These need to be displayed in reliable in vitro systems to comprehend a better understanding of the complex signaling pathways in vivo.

Poster Presentation (P-14)

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Metabolic activities of frozen/thawed human embryos under different oxygen conditions

The vitality of human embryos after vitrification is very important for final success of all cryoembryotransfer procedure. Optimization of cultivation conditions after thawing can support their regeneration.
In this study the thawed human morulae embryos (n 43) were cultivated for 24 hours in individual 25 µl drops in commercially available GTL medium (VitroLife) under condition with either 5% (n 21) or >20% (n 22) oxygen (O_2) in the atmosphere before their embryotransfer – transfer to the uterus. The metabolic activity of the embryos was determined by measuring the amino acids content of the spent culture medium by micellar electrokinetic chromatography with fluorescence detection. In the presence of 5 % O_2 was observed significant increasing of glutamic acid against 20 % O_2 atmosphere. In glutamin was significant decrease in 20 % O_2 in contrary of 5 % O_2. Both these amino acids are important for scavanger systems protecting cells against oxidative

phosphorylation. Also in tyrosine (amino acid with antioxidant effect) was observed significant decreasing/consumption in 20 % O_2 in compare 5 % O_2.
The results of these experiments suggest that the oxygen level affect the metabolic activity of the human embryos. During our experiments we observed very individual differences in metabolic activity between embryos from individual patients but results described above were common for all analysed embryos.

Oral Presentation (O-15)

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Dynamic landscape of peri-cenromeric heterochromatin during transition from naïve to primed pluripotency

Pluripotency, defined as the potential to generate all cellular lineages, is a continuous process starting in the inner cell mass (ICM) of the blastocyst and ending up with gastrulation. In the ICM, pluripotent cells are in a naïve state, characterized by an open chromatin conformation, with poor epigenetic controls. Then these cells acquire the competency to differentiate, along with compaction of chromatin, de novo DNA methylation and reshuffling of repressive marks on gene promoters. In such highly changing context, we have studied the fate of the constitutive, peri-centromeric heterochromatin (PCH) in pluripotent cells. We show that it switches from H3K27me3 enrichment to H3K9me3/DNA methylation enrichment, while the transcription status of the repeated sequences that composed PCH sharply decreases. This dynamic pattern appears as a faithful marker of the transition from naïve to primed pluripotency as it happens both in vitro in the different pluripotent cells that mimic both states (ESC and EpiSC, respectively) and in vivo within the late inner cell mass.

Poster Presentation (P-33)

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The analysis of neurodevelopmental disorders by the generation of 3D cerebral organoids mimicking the human cerebral development.

Background: Neurodevelopmental diseases such as schizophrenia are multifactorial, but a key component is the disturbed development of neurons. 3D organoids provide a powerful tool to study cells within 3D microenvironment. Defective neural development can be reflected by the disturbed growth, migration, motility or maturation of cells.
Methods: We used patient-specific induced pluripotent stem cells (iPS cells) for the generation of 3D cerebral organoids. Transcript and protein analysis verified the differentiation of mature neuronal and glial cells. Organoids were cut into sections for the immunofluorescence analysis suitable to detect aggregation and patterning of cells. The analysis focused on GABAergic cells.
Results: Early differentiation revealed the induction of early neural markers including SOX2 and PAX6. Neural induction towards cortical neurons and glia was monitored by a set of

neurodevelopmental marker genes including NESTIN, GFAP and NGN3. Analysis revealed a layer-like expression pattern for several mature makers including TBR1. The induction of GABA and its related molecular machinery pointed towards the differentiation into cortical GABAergic cells.
In conclusion, organotypic 3D disease models provide a powerful tool for the analysis of neurodevelopmental disorders.

Oral Presentation (O-39)

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Simple method of creating microfluidic structures using 3D printing

Assisted reproductive techniques (ARTs) employed in fertility clinics involves mainly hormonal hyperstimulation of ovaries, egg retrieval, standard insemination or intracytoplasmic sperm inection (ICSI) and culture of embryos until the time of transfer into the uterus. When fertilisation occurs naturally, a zygote is surounded by constantly changing dynamic environment, which helps the embryonic development. So far researchers have been trying to mimic the oviduct environment in in vitro condition for better understanding of the essential embryotrophic components of the oviduct fluid and their interactions with the embryo. The use of 3D printing technology to mimic the structures of in vivo oviduct can give interesting insights into mechanisms related to the early embryonic development and successfull pregnancy. In the

current study we aim to create a more in vivo like habitat in order to explore the differential effects of physical and structural origins on in vitro embryo development. 3D printing technology offers a simple method for fabricating patterns of various shapes and sizes. Here we use it for printing biocompatbile scaffolds and evaluate their fitness for creating microfluidic structures in polydimethylsiloxane (PDMS), a transpararent silicone gaining more and more attention in medical applications for its properties and handling simplicity. We compared the suitability of two different scaffold materials, one based on polivinyl alcohol (PVA) which is water soluble and one based on polylactic acid (PLA) which is not soluble in water, for simplified structure fabrication. This method provides an easy way to create biocompatible complicated structures of desired shape for medical applications.

Poster Presentation (P-44)

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Onapristone (ZK299) and mifepristone (RU486) regulate expression of membrane progesterone receptors in the bovine endometrium

Progesterone (P4) acts on uterus cell function by the nuclear P4 receptor (PGR) and membrane P4 receptor: progesterone membrane receptor component (PGRMC) 1 and 2, and membrane progestin receptor (mPR) α, β and γ. We have found earlier the variable expression of mRNA and protein for PGRMC1 and 2, PGRMC1 binding partner – SERBP1 (serpine 1 mRNA binding protein) and mPR α, β and γ in bovine endometrium during the estrous cycle, but the regulatory mechanism of mRNA expression of these receptors in endometrium is not clear. The aim of this study was examination weather P4 and its antagonists: onapristone (ZK299) and mifepristone (RU486) regulate of membrane P4 receptors mRNA and protein expression in cow endometrium. Endometrial slices from days 6-10 and 17-20 (n=4/per phase) of the estrous cycle were treated for 6 h (for Real Time PCR) and 24 h (for Western blot) with P4, ZK299 and RU486 (10^{-4}, 10^{-5}, 10^{-6}M each) for mRNA and protein expression analysis.

Progesterone (10^{-4}M and 10^{-6}M) inhibited ($P<0.01$) PGRMC1 mRNA expression on days 6-10 of the estrous cycle, and in dose 10^{-4}M on days 17-20. ZK299 (10^{-5}M and 10^{-6}M) and RU486 (10^{-4}M and 10^{-5}M) decreased ($P<0.05$) expression of mRNA for PGRMC1 only on days 6-10 of the estrous cycle. Moreover, P4 (10^{-4}M and 10^{-5}M) decreased ($P<0.05$) mRNA mPRα expression on days 6-10, however on days 17-20, all doses of P4, ZK299 and RU486 inhibited ($P<0.01$) mRNA mPRα expression. Studies factors did not affect ($P>0.05$) the PGRMC2 gene expression. Moreover, there were no effect ($P>0.05$) of P4 and used antagonist of P4 on level of proteins for membrane P4 receptors.
These results suggest that P4, ZK299 and RU486 may regulate PGRMC1 and mPRα mRNA expression in the bovine endometrial slices in a day and dose-dependent way and this way may influence on the endometrium function during the estrous cycle.

Oral presentation (O-16)

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Mesenchymal stem cells in self-assembled spheroids

Contemporary regenerative medicine holds great promise in mesenchymal stem cells (MSCs) use, particularly since this stem cells population enables patient specific treatment without ethical issues. However, the outcome of clinical trials involving MSCs didn't approve initial enthusiasm. These evidences strongly indicate importance to reconsider standard 2D adherent culture conditions for MSCs. Recent advancements in cell culturing include spheroids as a technique for 3D MSCs maintenance and expansion. Moreover, MSCs spheroids could also serve as cellular building blocks for tissue engineering. Thus, appropriate 3D culturing method could be used to obtain MSCs with characteristics more suitable for cell therapy application. Nevertheless, understanding of MSCs behavior in aggregates is in its beginning and results are quite elusive. Our preliminary data indicate the dependence of spheroid formation on MSCs tissue origin. Moreover, our ongoing research is trying to reveal changes in MSCs behavior following the 3D assembly. So far, we established optimal conditions for MSCs spheroid formation including cell density and time duration.

Within the current project, we are studying MSCs features after 24h of self-assembly using low adherence plates. In our focus are cytoskeletal modifications, as well as expression of MSCs and pluripotency markers changes and subsequent correlation with MSCs proliferative and differentiation ability.

Oral Presentation (O-2)

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Post-translational α-tubulin modification in 3D spheroids of rat glioma cells developed in transparent liquid marbles and hanging drops

Microtubules (MTs) are cytoskeletal component involved in many cellular events playing a crucial role in cell division. For these reasons they are a highly attractive target for anticancer drug design. During the cell replication there are several post-translational modification (PTMs) of MTs that have been considered as markers of tumor plasticity. Many studies on tumor cells has been used the 2D culture systems that do not exactly represent what happen in living organisms. A 3D model mimics the in vivo relevant physiological conditions allowing to better understand signaling between cells. In this work, spheroids from rat glioma cells (RGC) have been developed using the liquid marbles (LM) and the hanging drop (HD) technique. The former was assembled by rolling culture medium containing a suspension of single cells (30 µl/volume) on a bed of fumed silica nanoparticles. Two cell concentrations of RGC (5,000 and 15,000) were used. Control group were HD with the same volume and cell concentrations. After 24 and 48h of culture, cell behavior was observed by inverted microscope

and both tyrosinated(Tα-t) and acetylated α-tubulin(Aα-t) were evaluated in fixed spheroids by fluorescence and Western blot. RGC spontaneously formed spherical agglomerates, more rapidly in LM compared to HD system. The cell density influenced the size of spheroids. Larger size (> of 300 μm Ø) occurred with 15,000 cells compared to 5,000 cells (150 μm Ø).
Moreover, increase of Tα-t and Aα-t was observed in both HG and LM system from 24 to 48h($p>0.05$), with the highest values in 48h/LM spheroids of 5000 cells 5,000($p<0.05$). In conclusion, we showed that RGC cells cultured in LM express levels of PTMs of MTs that can be virtually used to evaluate the efficacy of new anticancer therapies.

Oral Presentation (O-46)

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Circularization of genes and chromosome by CRISPR

The CRISPR-based gene editing technologies have revolutionized biomedical research during the last few years. Using CRISPR gene editing, precision gene deletions, insertions, translocations, insertions or replacements have been successfully introduced into a broad variety of cells and organisms. Extrachromosomal circular DNA (eccDNA) and ring chromosomes are genetic alterations found in humans with genetic disorders. However, there is a lack of genetic engineering tools to recapitulate and study the biogenesis of eccDNAs. Here, we created a dual-fluorescence biosensor cassette, which upon the delivery of pairs of CRISPR/Cas9 guide RNAs, CRISPR-C, allows us to study the biogenesis of a specific fluorophore expressing eccDNA in human cells. We show that CRISPR-C can generate functional eccDNA, using the novel eccDNA biosensor system. We further reveal that CRISPR-C also can generate eccDNAs from intergenic and genic loci in human embryonic kidney 293T cells and human mammary fibroblasts. EccDNAs mainly forms by end-joining

mediated DNA-repair and we show that CRISPR-C is able to generate endogenous eccDNAs in sizes from a few hundred base pairs and ranging up to 207 kb. Even a 47.4 megabase-sized ring chromosome 18 can be created by CRISPR-C. Our study creates a new territory for CRISPR gene editing and highlights CRISPR-C as a useful tool for studying the cellular impact, persistence and function of eccDNAs. In this presentation, the CRISPR-C technology, as well as a few other CRISPR technologies and applications, will be presented at the COST CellFit annual meeting.

Ref:

Henrik Devitt Møller, Lin Lin, Xi Xiang, Trine Skov Petersen, Jinrong Huang, Luhan Yang, Eigil Kjeldsen, Uffe Birk Jensen, Xiuqing Zhang, Xin Liu, Xun Xu, Jian Wang, Huanming Yang, George M. Church, Lars Bolund, Birgitte Regenberg, Yonglun Luo. **CRISPR-C: Circularization of genes and chromosome by CRISPR in human cells.** Nucleic Acids Research. https://academic.oup.com/nar/advance-article/doi/10.1093/nar/gky767/5078801

Oral Presentation (O-17)

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Mimicking the maternal environment to optimize the in vitro production of embryos in wild and domestic mammals

A wide range of reproductive biotechnologies are now available to facilitate the conservation and genetic management in mammals. The maternal environment offers optimized conditions for fertilization and embryo development, based on a constant dialogue between somatic and germinal tissues. In deer species, it has been clearly shown that the presence of oviduct epithelial cells (OEC) can improve the rate and quality of embryo development in vitro. In bovine, we showed that this beneficial effect of OEC relies on a molecular dialogue with early developing embryos, modulating the gene expression profiles of both partners. In pigs, we showed that oviduct fluid can regulate the polyspermy, which is a major problem for IVP in this species. Recently, extracellular vesicles such as exosomes emerged as a new paradigm in cell communication. These membrane vesicles of 50-150 nm can transport proteins, mRNA and ncRNA from secreting cell to target cells and modify their properties. We found exosomes in OF, containing proteins and different RNAs, including microRNA and we showed that these exosomes are able to reproduce the effects of

OEC on embryo development in cattle as well as the effect of OF on pig IVF.

Oral Presentation (O-18)

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Priming hnMSCs' secretome using 3D spheroid cultures for the treatment of cutaneous wounds

Cutaneous wound healing consists of a complex series of events which success is impaired in many chronic diseases. The current available therapeutic options fail to promote full tissue regeneration and mesenchymal stem cell (MSC)-based therapies have emerged as promising alternatives. The use of 3D culture models has gained relevance as a strategy for priming cells to a more healing-prone secretome. This study aimed at evaluating the impact of 3D cultures on the secretome of human neonatal MSCs (hnMSCs) and its therapeutic potential for wound healing. Conditioned medium was prepared from hnMSCs cultured as spheroids (CM3D) and in monolayer (CM2D) and its content assessed through proteomic analysis. Both CM (concentrated 0.5–10x) did not induced cytotoxicity in keratinocytes (HaCaT) and dermal fibroblasts (HDF). In turn, CM3D 10x enhanced HDF proliferation by 40%, whereas CM2D showed no effect. Furthermore, CM3D significantly enhanced elastin production and migration of HaCaT and HDF in vitro. In an rat wound healing in vivo

model, macroscopic observations showed that CM3D (10x)- and CM2D (10x)-treated wounds closed at 12 and 13 days after injury, respectively. Histological analyses, at these time points, revealed that only CM3D-treated wounds showed an advanced tissue resolution with higher levels of re-epithelialization and granulation tissue formation. Overall, the results indicate that 3D cultures enhanced the therapeutic potential of hnMSC secretome which may constitute a novel cell-free-based therapy for skin regeneration.

Oral presentation (O-35)

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Liquid pearls for anticancer drug discovery

Antitumor efficacy, in 2D models, does not translate faithfully to patient outcome, as 3D cell culture is today the key tool for screening platforms. We propose to create and evaluate a new high content screening assay based on liquid pearls (LP): micro-tumors liquid marble incubators will be fused with other containing endothelial and/or immune cells. Generated multi-phenotypic LPs will allow us to compare the influence of 3D co-cultures to the mono-phenotypic cultures strategy results previously obtained in 2D and 3D.
Such a co-culture of different phenotypes (immune, vascular, hepatic) mimicking the complexity of an in vivo tumor tissue with micro-environmental signals, will undoubtedly lead to a better patient outcome when a new putative anticancer drug is identified by that way.

Oral Presentation (O-19)

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Bioengineering the Structure of the Bovine Endometrium Using Advanced Cell Culture technology

3D cell culture systems that resemble the structure of real tissues may provide in vitro models to study cellular responses. Currently, 3D endometrial in vitro systems may allow: 1/ studying physiology and pharmacological aspects of the endometrium; and 2/ reproducing the micro-environment where the early embryo develops.

The aim of this study was to establish long term culture of bovine stromal cells on a synthetic polymer scaffold (Alvetex ®).

Bovine frozen-stored stromal cells isolated from slaughterhouse uteri (1) were thawed and cultured on Alvetex scaffold following guidelines provided by manufacturer. Cell cultures were fixed at 3 time points (D7, D13 and D21) for histomorphometry and immunohistochemistry (IHC) analysis (1).

Upon examination of cross sections stained with haematoxylin-eosin, patterns of stromal cell (SC) growth were established. Thus, on D7 of culture, SC isolated during the follicular phase (FF) of the oestrus cycle penetrated homogenously into the scaffold whilst SC isolated during late luteal phase (LP) grew

preferentially on the upper and lower surface of the scaffold. By D21, LP-SC masses appeared larger within the scaffold, but they did not penetrate.

FF and LP stromal cell origin was confirmed by positive staining with vimentin and morphology throughout.

Additionally, the presence of extracellular matrix (ECM) protein, collagen I, was assessed by IHC. Interestingly LP-SC secreted collagen I on top of Alvetex scaffold, with particular strong expression at the edges of the device. However, FF-SC did not express collagen I.

The present report demonstrates that Alvetex scaffolds can support bovine endometrial stromal culture for long periods. The stromal layer of endometrial fibroblasts will serve as a foundation to grow endometrial epithelial cells.

Furthermore, the robust expression of collagen I by LP-SC suggests that epithelial cells might be seeded on top of the stromal compartment without heterologous ECM components.

(1) Súarez M et al., Ceramics International. 2018; 44:14920–14924.

Poster Presentation (P-21)

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Bovine embryo selection by single blastomere biopsy and gene expression analysis

Selecting high quality in vitro produced (IVP) embryos for transfer is a difficult task when selecting embryos only by visual observation. This study was conducted to see differences in gene expression profiles from biopsied IVP bovine morulae. On day 5, one blastomere was aspirated with a microneedle from 20 morulae from which thereafter 12 (60%) were arrested in morula stage (group A) and 8 (40%) developed into blastocysts (group B). cDNA was synthesized using the Ovation RNA-Seq System V2 Kit (Nugen) and sequenced using SOLiD 5500 Wildfire platform. Out of 108 760 successfully mapped genes, 1204 showed a difference in mRNA expression level with a p-value $<10^{-5}$. Out of these, 155 genes were up-regulated in embryos developing to blastocysts. The pathway enrichment analysis of embryos developing to blastocysts revealed significant enrichment in “organelle biogenesis and maintenance”, “mRNA splicing” and “mitochondrial translation” pathways with p-value < 0.015. Our findings suggest principal differences in gene expression patterns and functional networks of embryos able to reach the

blastocyst stage compared to embryos arrested in development. This technique allows embryo selection at an early developmental stage without compromising embryo production.

This study was supported by Enterprise Estonia grant EU30020, Institutional research funding IUT 8-1 and Horizon 2020 Project SEARMET 692299.

Oral Presentation (P-49)

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3D tissue engineered cartilage grafts for the repair of patellofemoral osteochondral kissing lesions

Osteochondral injuries include damage of articular cartilage and adjacent subchondral bone. They can occur in any joint but are most common in the knee. Osteochondral “kissing lesions” are still untreatable and can lead to the development of osteoarthritis. Kissing lesion in patellofemoral joint involves lesion on the patella and trochlea that are in the direct contact with each other. In our study we wanted to compare two types of autologous nasal chondrocyte-based tissue engineered cartilage grafts that are used for the treatment of patellofemoral osteochondral kissing lesion in a sheep model.
Nasal chondrocytes (NC) were isolated from sheep nasal septum cartilage biopsy and expanded in monolayer for 13 days. Collagen scaffold (Chondro-Gide) was used to provide 3D environment for the cells. Two types of cartilage grafts were manufactured: N-CAM (immature graft with pre-cultivation of 2 days) and N-TEC (mature grafts with pre-

cultivation of 2 weeks). Manufactured grafts were embedded in paraffin and stained with HE, Safranin O and immunostained using antibodies directed against collagen type II and collagen type VI.
N-TEC grafts contained higher amount of glycosaminoglycans (GAG) compared to the N-CAM grafts. Collagen type II was expressed only in N-TEC grafts. Collagen type VI was localized pericellulary in N-TEC grafts while in N-CAM grafts it was present throughout extracellular matrix.

Funding for this research has been received from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 681103, BIO-CHIP.

Oral Presentation (O-23)

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Regeneration of kissing cartilage lesions in the knee using autologous nasal chondrocyte-based cartilage grafts

Complex cartilage lesions called "kissing lesions" are defined with the presence of lesions in direct contact with each other. Clinical results of cartilage repair techniques have been shown to be worse in cases of kissing lesions as compared to non-kissing lesions. A sheep animal model was designed to test the possibility of kissing cartilage lesion regeneration using nasal chondrocyte-based autologous cartilage grafts. A kissing lesion was created in the medial part of the patellofemoral compartment of the right stifle joint. 6 mm osteochondral defects (2-3 mm in depth) were made on the articular surfaces of the femoral trochlea and patella using a drill bit. Two types of cartilage grafts were engineered: N-CAM (immature graft with little or no extracellular matrix) and N-TEC (mature grafts with extracellular matrix containing cartilage specific proteins). Engineered cartilage grafts were characterized with molecular and histological methods. The grafts and collagen membrane were trimmed to an appropriate size with a skin biopsy punch and implanted into the defects using a press-fit method. The study included three test groups (N-CAM, N-TEC and control group) with two time points (6 weeks and 6

months). After euthanasia, the osteochondral tissue blocks comprising treated defects and surrounding tissue was explanted and analyzed by gross examination, MRI, histology and immunohistochemistry. Here we present preliminary results from an ongoing study. Nasal chondrocyte–based cartilage grafts shows promising results in repair of damaged articular cartilage.

Funding for this research has been received from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 681103, BIO-CHIP.

Poster Presentation (P-24)

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Culture models for embryonic diapause in roe deer

Embryonic diapause is a temporal reduction of developmental pace at the blastocyst stage. Diapause was first described in the roe deer (Capreolus capreolus)[1] and by now has been described in over 100 mammals[2]. Yet, the molecular mechanisms regulating diapause are still poorly understood. In the roe deer, fertilization takes place in July or August. Until mid December blastocysts remain in the uterus showing only marginal growth (<3mm). Embryo-maternal communication during diapause solely occurs via the histotroph. To investigate embryo-derived signals, we aimed at culturing embryos in a defined medium, later to be analysed by mass-spectrometry. We collected uteri from roe deer shot during huntings in autumn 2017. Embryos were flushed, washed and cultured for 24h. Apart from a reduction in estimated embryo size (-26 ± 16%), there were no morphological changes after culture. However, only 3 out of 25 embryos remained viable. Embryos stained directly after collection from uteri already showed low viability. A potential reason could be the late sampling of the embryos after death of the animals. Other sampling approaches would lower the number of embryos to be analysed, making analysis of the

embryonic secretome rather unfeasible. To distinguish the origin of signalling factors in the histotroph, we thus will aim at analysing the endometrial secretome. Yet, endometrial cells rapidly change their morphology and physiology in standard 2D culture. Therefore, we currently aim at developing a suitable 3D culture system that allows preserving the physiological state of endometrial cells.

1) Bischoff, T. L. W. Entwicklungsgeschichte Des Rehes. Kessinger Publishing, (1854).
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Poster presentation (P-25)

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Increased abundance of pro-inflammatory cytokines in EV from high somatic cell count milk

The role of extracellular vesicles (EV) during the course of mastitis in dairy cattle is largely unknown. Differences in EV cargo, specifically concerning proteins and miRNA, have been described in milk from cows with mastitis, characterized by a high somatic cell count (SCC). Until now, a study focusing on the mRNA cargo of milk EV is lacking. The aim of this study was to evaluate whether an increase of the milk SCC affects the mRNA abundance of cytokines in milk EV. Milk from eight Brown Swiss dairy cows was collected individually from each quarter and the SCC was determined. Then, using 100.000 cells/ml as a threshold, samples were distributed into low SCC and high SCC experimental groups. The average cell number in the low SCC group was 22’179 ± 21’666 cells/ml, while for the high SCC group was 602’833 ± 609’297 cells/ml. EV were isolated from skimmed milk using filtration and differential ultracentrifugation. The presence of EV was confirmed by transmission electron microscopy (TEM) and western blot (WB) for the presence of both TSG101 and CD81. In addition, the absence of Calnexin protein as determined by WB

confirmed the absence of intracellular contamination. The transcriptional abundance of specific key cytokines involved in udder immune response (TNFα, IL-8, IL-10, IFNγ and MyD88) was determined by qPCR. IL-1β, IL-10 and IFNγ were expressed below the detection level.
While MyD88 transcripts were not altered between high and low SCC groups, the abundance of IL-8 and TNFα mRNA was higher in milk EV from high SCC. Our results showed that milk EV cargo changes regarding specific cytokine mRNA during a mammary gland inflammation, pointing towards an immunoregulatory role of EV during mastitis.

Poster Presentation (P-37)

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Co-authors:
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Devising a macromolecular crowded system for shaping the endometrial cell culture microenvironment

Modelling the interactions between the maternal endometrium and the embryo has challenged scientists to improve in vitro systems for its study. As the endometrium replenishes itself cyclically, new extracellular matrix (ECM) is assembled. Additionally, during decidualization the endometrial ECM is remodelled to create the adequate environment for embryo implantation.
The ECM is an essential basic component of all tissues and organs which apart from giving structural support, promotes tissue functionality by inducing cell differentiation, proliferation and migration. To mimic the biological function of the ECM *in vitro,* artificial polymer scaffolds, gels or coatings with ECM components have been introduced to cell culture models. These artificial scaffolds, however, do not fully confer ECM biological activity. One possible way to fully confer ECM biological activity is using macromolecular crowding. Macromolecular crowding allows cells to recreate their microenvironments and improve cell functionality by concentrating the availability of substrates in the culture media,

and enhancing the capacity of protein folding and ECM assembly.
To test whether macromolecular crowding of endometrial epithelial cell (EEC) microenvironment would influence the capacity of ECM assembly and cellular function, we examined receptive and non-receptive EEC. We compared ECM deposition, expression profile of endometrial markers and interactions with trophoblast cells. We will describe the behaviour of the two different EEC lines under crowded environments. The ability to alter the cell molecular microenvironment will allow us to improve our understanding of the endometrium-embryo interactions during embryo implantation.

Oral Presentation (O-47)

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Scaffold micro-patterning for peripheral nerve regeneration: *in vivo* studies

Several bioengineering approaches have been proposed for peripheral nervous system repair, with limited results and still open questions about the underlying molecular mechanisms. The micro-patterning, mechanical properties and the degradation rate of collagen-based scaffolds can be conveniently tailored to the tissue of interest, allowing the optimal tissue-material interaction. We assessed the biological processes that occur after the implantation of collagen scaffold with a peculiar porous micro-structure in a rat sciatic nerve transection model. It was compared to commercial collagen conduits and nerve crush injury using functional, histological and genome wide analyses. We demonstrated that within 60 days, micro-patterned scaffold had been completely substituted by a normal nerve. Gene expression analysis documented a precise sequential regulation of known genes involved in angiogenesis, Schwann cells/axons interactions and myelination, together with a selective modulation of key

biological pathways for nerve morphogenesis induced by porous matrices. These data highlight the significant influences of an instructive micro-environment on cell behaviors to enhance nerve morphogenesis. This can be exploited to improve recovery and understand the molecular differences between repair and regeneration. A human *phase I* study protocol for sural nerve regeneration after biopsy has been approved.

Poster Presentation (P-26)

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(presented by Alice Jouneau and Anne Couturier-Tarrade)
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A new culture model of rabbit trophoblastic cells to explore cell function and transplacental transfers

The placenta controls exchanges between the mother and the fetus and therefore fetal development and growth. The maternal environment (nutrition, exposure to pollutants...) can lead to disturbance of placental functions, with consequences for the health of the offspring. To limit the use of animal experiments to study transplacental transfers and to investigate the mechanistic aspects of placental function, we are developing a cell culture model to mimic the placental barrier. Since the rabbit placenta is closest to that of humans, rabbit experiments can provide biomedical data regarding human placental function. Thus, our cellular model uses rabbit trophoblastic cells, which allows to compare in vitro data to results from in vivo experiments in rabbits. To work with cells close to primary cells, we chose to derive trophoblastic stem cells from rabbit blastocysts and to differentiate them into mature

trophoblastic cells. In particular, these cells are cultured in the presence of a flow of medium, that promotes the appearance of microvilli on the cell surface, the fusion of cytotrophoblasts into syncytiotrophoblasts and the formation of lipid droplets. The cell transcriptome is being characterized. Thereby, the culture model allows mimicking the in vivo conditions in which maternal blood flow exerts mechanical forces on trophoblastic cells and influences their phenotype.

Oral presentation (O-48)

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Nanotopographies transmit physical information to the genome through modulation of the nuclear lamina

Cells interacting with engineered biomaterials respond to topographical cues on the nanometer scale. We found that nanotopographies can directly affect the architecture of the nuclear envelope, in addition to the cytoskeleton and cell-surface adhesion complexes. Changes in the mechanical properties of the nucleus in turn affect gene expression and cell behaviour, such as migration and proliferation. In cells cultured on micron-sized Si needles with nanometer-scale tips, lamin A/C, but not lamin B1, was recruited to sites of nanoneedle impingement. LMNA gene expression also increased in cells grown on nanoneedles whereas total lamin A levels decreased, suggesting a higher rate of protein degradation. Lamin A expression has been shown to increase with substrate stiffness in order to protect the contents of the nucleus from mechanical stresses and it plays an important role in stem cell differentiation. To further investigate the role of lamin A in transducing topographical signals, nuclear lamins were depleted by RNAi and cell growth, proliferation, and gene

expression on nanoneedles were measured. These studies demonstrate that the spatial organization of lamin A is critical for sensing and responding to nanometer-scale physical forces.

Poster presentation (P-38)

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Fine tuning of implantation: how small molecule dynamics play a big role in cell-cell communication

Successful pregnancy is dependent on a succession of discrete events. An early critical stage is implantation, when the blastocyst adheres and invades the uterus. For implantation to succeed, a synchronized network of molecular interactions between the uterine endometrium and the blastocyst must occur. This cross-talk is mediated by a range of molecules such as hormones, cytokines, chemokines and integrins. The receptive endometrial cells acquire specific phenotypical features during a narrow time of the menstrual cycle termed the window of implantation.
Embryo implantation failure is a major obstacle in the field of assisted reproduction. Embryonic and maternal deficiencies, such as in endometrial receptivity, could hinder the cross-talk between them. Although key molecules have been identified, we lack comprehensive understanding of the early dynamics of implantation. To examine the early sequence of events that fine tune the embryo-endometrium cross-talk we use receptive and non-receptive human endometrial cell lines and trophoblast spheroids as an *in-vitro* model, and various molecular and

imaging approaches to monitor the temporal dynamics of intercellular interactions.
Studying the dynamics of the cross-talk, and specifically signals that promote implantation at early stage, will shed more light on the implantation process and possibly guide new strategies in diagnosing and preventing implantation failure.

Poster Presentation (P-27)

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Characterizing bovine uterine fluid during the window of conceptus elongation.

Embryo mortality is a significant contributor to poor reproductive efficiency in cattle, with most losses occurring during the first 2-3 weeks post-conception. Successful pregnancy establishment is contingent on conceptus (embryo and extra-embryonic tissue) elongation, which commences ~Day 13, and is independent of pregnancy recognition, which occurs ~Day 16. Moreover, elongation is driven by P4-induced changes in the uterus. Our aim was to identify the metabolic signals driving and sustaining conceptus elongation. We analysed the composition of uterine luminal fluid (ULF) on Days 12, 13 and 14 of the oestrous cycle from synchronised crossbred beef heifers, either treated with an intravaginal P4-releasing intrauterine device from Days 3 to 6 (high P4, n=20), or not (normal P4 control, n=15). ULF was collected as previously described [Forde et al. (2014) Plos one 9(6):e100010] and screened of over 1000 metabolites using high-throughput liquid chromatography tandem mass spectrometry (LC/MS/MS) [Evans et al. (2014) Metabolomics

4(1):1000132]. To date, this ongoing study has identified 221 metabolites, of which 36.2% are involved in amino acid metabolism, 32.1% in lipid metabolism, 9% in nucleotide metabolism, and 8.6% in carbohydrate metabolism. In addition, 5% of constituents are xenobiotics, 4.1% cofactors and vitamins, 3.6% energy substrates, and 0.9% peptides.

These data are important for (a) understanding the microenvironment evolved to facilitate elongation – a fundamental developmental phenomenon which cannot currently be recapitulated in vitro, and which coincides with the period of greatest pregnancy loss in cattle, and (b) serving as an in vivo reference of uterine fluid composition for assessing the relative merits of future in vitro uterine models.

The comprehensive analysis of these metabolites, in addition to testing these compounds in in vitro embryo culture to determine the biological significance of our observations, are areas of ongoing research.

This work is supported by Science Foundation Ireland (SFI: 13/IA/1983), Irish Research Council (IRC: GOIPD/2017/942), and University College Dublin (UCD: CDA54580).

Poster Presentation (P-28)

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Influence of FSH, LH, PRL and GH on AQP1 expression in the porcine ovarian follicular cells

Aquaporins (AQPs) are channels integrated with the cell membranes and play a significant role in regulation of water homeostasis. The present in vitro study analyzed whether the hormones that affect the ovarian follicular steroidogenesis process also participate in the regulation of AQP1 mRNA and protein expression. We examined the effect of FSH, LH, PRL and GH on AQP1 expression in granulosa (GC) and theca cells (TC) in monolayer culture and co-cultures. Granulosa and TC were isolated from middle and large ovarian follicles of gilts and were incubated for 24 hours with experimental factors. Gene and protein expression of AQP1 was evaluated by real-time PCR, Western blot and immunofluorescence, respectively. The results showed that FSH increased expression of AQP1 in GC from middle and large follicles in relation to control. In TC from middle follicles PRL stimulated the expression of AQP1. It was also found that AQP1 protein was detectable in TC isolated from large porcine follicles cultured with LH.

AQP1 protein was observed in cytoplasm, endosoms and membrane of GC and TC from middle and large follicles in control and after treatment with all studied factors. In co-cultures increased expression of AQP1 was observed in GC from middle follicles incubated with FSH in relation to control, however in co-cultures of TC from middle-sized follicles there was indicated by LH and GH.

Significantly increased expression of AQP1 was observed in co-cultures from GC of large follicles by GH. These present results have provided some novel insights into the regulation of AQP1 present in GC and TC of porcine ovarian follicles, suggest that FSH, LH, PRL and GH may mediate the action of AQP1.

Poster Presentation (P-1)

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Pituitary hormones (FSH, LH, PRL and GH) differentially regulate AQP5 expression in the porcine ovarian follicular cells

Aquaporins (AQPs) are proteins integrated with the cell membranes that form selective channels to water and other molecules. The aim of the present study was to examine the effect of FSH, LH, PRL and GH on AQP5 expression in granulosa (GC) and theca cells (TC). Granulosa and TC were isolated from middle and large ovarian follicles of mature gilts. The monolayer cell culture and co-cultures were incubated for 24 hours with experimental factors. Gene and protein expression of AQP5 in the ovarian follicles cells was evaluated by real-time PCR and Western blot, respectively. The results showed that GH significantly decreased expression of AQP5 in GC from middle follicles in relation to control. In GC from large follicles PRL stimulated the expression of AQP5. However, increased expression of AQP5 in TC from large follicles was indicated by GH and PRL in relation to control. Significantly higher expression of AQP5 protein in GC from middle and large follicles was indicated by FSH and PRL in

relation to control. In co-cultures increased expression of AQP5 was observed in GC from large follicles incubated with LH, PRL and GH versus control. Significantly increased expression of AQP5 was observed in co-cultures of TC from all type of follicles incubated with LH, whereas PRL stimulated expression of AQP5 in TC from middle-sized follicles. The present studies delineated the specific AQP5 expression patterns in response to FSH, LH, PRL and GH and indirectly provided novel evidence for the role of AQP5 in maintaining local fluid balance within the follicular cells in pigs.

Poster Presentation (P-4)

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Genotoxic activity of PhIP (2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) evaluated in a liver 3D cell model

Human hepatoma HepG2 cells cultured in two-dimensional (2D) monolayers are one of the most often used experimental systems for investigating the adverse effects of xenobiotics in vitro. However, most hepatic cells in 2D lack relevant hepatic properties, mostly due to limited expression of drug metabolizing enzymes, which makes the extrapolation of the results to in vivo conditions questionable. Cells in 3D, as compared to 2D, have enhanced cell-cell and cell-matrix interactions, they can better replicate intrinsic conditions and cellular responses in vivo to external impacts and they express higher liver-specific functions including metabolic enzymes. The aim of the study was to develop a 3D cell culture model with HepG2 cell line and to validate the model using indirect-acting model genotoxic compound, PhIP (2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine). The 3D cell cultures were prepared with a forced floating method, cultured for three days and then exposed to non-cytotoxic concentrations of PhIP (50,

100 and 200 µM) for 24h. PhIP induced DNA damage in 3D but not in 2D cell model detected with the comet assay. Furthermore, the up-regulation of the expression of metabolic genes from phase I and phase II and DNA damage responsive genes was determined at the mRNA level (qPCR) and confirmed at the protein level (Western blot).

The results suggest that the newly developed 3D model with HepG2 cells has improved metabolic capacity compared to 2D model and has the potential for the application in genotoxicity studies as well as in the regulatory testing of new chemicals and products.

Poster Presentation (P-29)

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Possible effect of PUFAs on gelatinases and MMP-14 mRNA in rabbit differentiated adipose derived stem cells in vitro

The matrix metalloproteinases (MMPs) is a large family of zinc-dependent proteases that degrade extracellular matrix proteins and cell-surface regulatory proteins. There are reports suggesting dominant role of MMP-14 and MMP-2 in adipose tissue development in mice. In our previous study we found that PUFAs up-regulated mRNA expression of lipolysis associated genes in rabbit adipose derived stem cells (ADSCs). In respect to aforementioned data we aimed to explore the effect of two PUFAs (DHA and EPA) on the expression at mRNA level of genes encoding three MMPs in rabbit ADSCs. Methods: The total mRNA was obtained from subcutaneous and visceral ADSCs treated with 100 µg DHA or EPA for 48 hours. The expression of the target genes, MMP-2, MMP-9, and MT-MMP1 (MMP-14) was evaluated by SYBR-based real-time RCR using $\Delta\Delta Ct$ method with GAPDH as house-keeping gene. Results: We did not observed significant difference in the expression of the studied MMPs between any

of the cells subgroups, although there was a tendency for elevated levels of all studied MMPs in treated visceral cells. Moreover, EPA significantly enhanced the MMP-14 mRNA expression than MMP-9 in subcutaneous adipose cells (p=0.041).

Conclusions: The results of the current study do not show categorically a notable effect of EPA and DHA on MMP-2, MMP-9 and MMP-14 mRNA expression in rabbit subcutaneous and visceral adipose derived stem cells. However the observed changes suggest that those PUFAs might modify in some extend the transcriptional profile of MMPs in adipose tissue.

Guest Speaker (O-34)

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Evolution in 3D cell culture: synthetic hydrogel with unique biomechanical properties

The most commonly used matrices for 3D cell culturing are from animal origin. These matrices have batch-to-batch variation resulting in inconsistent results. Noviocell has synthetic hydrogels and develop them into easy-to-use solutions. The unique combination of tunable biofunctionality and biomechanics of these hydrogels make them excellent matrices for 3D cell culture: stem cells, organoid growth, personalized medicine or regenerative medicine.
Noviocell has two hydrogel technologies; a soft reversible thermo-responsive helical oligo(ethylene)glycol polyisocyanopeptides hydrogel (Noviogel) and a pH triggered self-assembly of peptide-modified cyclohexane triacid derivatives that self-assemble into nanofibers (Syntrix).
Noviogel is an unique fully synthetic biomimetic extracellular matrix (ECM) with almost identical biomechanical properties as natural matrices. The complex chemistry of the polymer allows organization into a helix-like structure that is similar to the conformation of collagen. The porous fibrous polymer network has a strain stiffness that increases under applied force. This creates a cellular microenvironment with the right mechanical cues to control cell expansion and differentiation.
Syntrix is a fully synthetic hydrogel that self-assemble rapidly and spontaneously under physiological conditions from

appropriate precursor solutions, allowing mild encapsulation of cells. A key feature is the ease and accuracy with which the surface of the fibres can be functionalized to present ligands. Mixing of hydrogel-ligand conjugates provides a single-step modular approach to generate diverse synthetic bioactive matrices approaching the complexity of hydrogel-like systems such as natural ECM.

Poster Presentation (P-30)

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Highly dynamic uterine fluid protein profile during early embryo development in the European roe deer (Capreolus capreolus)

Early embryo development is a highly dynamic process, in which the uterine environment adapts to the embryos' needs. Advances in in vitro culture systems greatly improved the simulation of the in vivo situation, but for optimal modelling, a full understanding of physiology is crucial. The roe deer fertilization and subsequent implantation are decoupled by a 4-month period of diapause during which embryo development is highly decelerated. Prior to implantation, development resumes a fast pace. This period was used to gain insight into proteome changes in the uterine fluid. Samples were collected during regular huntings from September to December, and four developmental stages were assigned based on the embryonic genomic DNA content: early, mid, late diapause, and elongation. The relative abundance of 819 identified and quantified proteins with a FDR <1% as assessed by LC-MS/MS

was compared, revealing 106 differentially abundant proteins (DAP) in the uterine fluid corresponding to diapausing and elongating embryos. The dynamic proteome changes were studied by a self-organizing tree algorithm. The DAP followed one of the following patterns: (1) a gradual decrease over development; (2) high abundance during early and mid diapause, low abundance during late diapause and elongation; 3) high abundance during diapause, low abundance during elongation; and 4) low abundance during diapause, high abundance during elongation.

The proteins with highest abundance during diapause included phosphoserine aminotransferase (amino acid biosynthesis) and uteroglobin (phospholipase A2 inhibition), while those with higher abundance during elongation included thioredoxin (redox signalling) and endoplasmic reticulum chaperone BiP (protein folding).

This study shows that during decelerated embryo development, the microenvironment continuously changes. This underlines the need for more specialized and dynamic in vitro embryo culture systems to resemble the in vivo system as best as possible.

Oral Presentation (O-20)

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Development of a methacrylated gelatin-based 3D Artificial Intestine platform

Farmed fish diet is largely based on fishmeal and fish oil. Consumer and environmental groups demand a continued move towards alternative feeds. The objective is to use plant-based feeds from sustainable agriculture. We are developing a 3D in vitro platform to predict the nutritional value of alternative feed and functional additives. To identify the scaffold material with the most suitable biomimetic surface properties, we have prepared methacrylated gelatin hydrogel films with A) high (93%) and B) low (66%) degree of substitution. These films were characterized by gel fraction test, swelling experiment and texture profile analysis (TPA). Biocompatibility was assessed by repopulating with mouse fibroblasts. Both A and B displayed a gel fraction higher than 90% and therefore a high stability. The degree of swelling was higher in A. TPA showed that both films have the same resistance to compression but the one with higher degree of substitution showed better cohesiveness, and recovery potential

behaviour. No differences were observed in fibroblast adhesion and proliferation rates. Future experiments will evaluate A and B permeability to metabolic relevant molecules, in order to select the most suitable scaffold material.

Acknowledgments: Supported by Carraresi Foundation.

Oral Presentation (O-31)

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Analysis of human neural stem cell secretome during neuronal differentiation in vitro

Neurodegenerative diseases (e.g. Alzheimer's, Huntington's disease) negatively affect life of approximately 17% of the world population and have significant socioeconomic impacts. One of the potential therapeutic avenues in these diseases is a transplantation of neural stem cells or differentiated neural cell populations. To develop better protocols for in vitro culture and differentiation of these cells, we need to improve the understanding of signaling pathways regulating these processes. Secreted proteins, including growth factors, cytokines and chemokines mediate intercellular communication and regulate cell survival, proliferation and differentiation.

We used model of spontaneous (FGF/EGF withdrawal) and induced (BDNF/GDNF supplementation) in vitro differentiation of human neural stem cells to monitor changes in secretion of selected factors using Luminex human 30-plex panel. The most prominent identified changes were an

increased secretion of VEGF by differentiated cells and changes in secretion of IL-6 and IL-13. Roles of these factors in induction or maintenance of differentiated phenotype deserve further studies. We currently follow up with the whole proteome LC-MS analysis of these cells.

Acknowledgements: This research was supported by the Czech Ministry of Education, Youth and Sports, projects LO1609 and LTC18079.

Poster Presentation (P-32)

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The effect of prostaglandin F2α on the human trophoblast-derived HTR-8/SVneo cell line

Prostaglandin F2α (PGF2α) is the main luteolytic factor. Hence, its exogenous administration can terminate pregnancy. On the other hand, elevated amounts of PGF2α in uterine lumen and/or its increased endometrial synthesis are observed during the implantation period in various mammals. The aim of our study was to determine if PGF2α regulates expression of genes and activity of proteins involved in tissue remodeling and trophoblast invasiveness (metalloproteinase 2 and 9; MMP2 and MMP9) in the human trophoblast-derived HTR-8/SVneo cell line. HTR8/SV-neo cells were incubated with vehicle or PGF2α (100 nM; 1 μM) for 24 h. In cell lysates, MMP9 gene expression was studied by real-time RT-PCR. Activity of MMP2 and MMP9 was measured in conditioned media by gelatin zymography. Protein expression of PGF2α receptor protein was determined by Western blot. The presence of PGF2α receptor protein was detected in the HTR-8/SVneo cells. PGF2α increased gene expression and protein activity of metalloproteinase 9 (1 μM; $P<0.05$) in HTR-8/SVneo cells.

PGF2α had no effect on activity of MMP2. Our findings suggest that PGF2α during embryo implantation is potentially involved in regulation of human trophoblast invasiveness.

Research supported by the basic grant of the Institute of Animal Reproduction and Food Research of the PAS.

Guest Speaker (O-45)

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3D tumorspheres as models to assess cellular responses to chemotherapy

Pancreatic cancer is a highly lethal disease. Despite improvements in diagnosis, treatment and surgical care, the overall 5-year survival is 8%. Surgery offers the best curative treatment, but < 20% of patients present with tumours eligible for resection at initial diagnosis. Aggressive invasion, early metastasis, formation of desmoplastic stromal reaction within the tumour and resistance to chemo/radiotherapy are the major causes of treatment failure and recurrence in pancreatic cancer. Cancer Stem Cells (CSCs) are a small population of cells responsible for tumour initiation, progression, metastasis and chemo-resistance. CSCs also have been considered as the main cause of cancer recurrence. Therefore, targeting CSCs could be an effective strategy to improve the survival outcomes of pancreatic cancer patients. Previously, we found increased expression and secretion of the CSC marker *ALDH1A1* in highly invasive cells compared to low invasive cells using proteomic profiling. However, current 2D cell culture platforms often fail to recapitulate the physiology of the tumour microenvironment due to the different architecture and biochemical gradients. My research uses 3D culture of pancreatic cancer PDX-organoids and cancer cell line tumoroids to recapitulate the multicellular architecture of the

original tumour. We show that pancreatic cancer 3D tumorspheres are intrinsically resistant to chemotherapeutic drugs with CSC-related markers increased in the 3D tumorspheres. Further profiling of the therapeutic resistant 3D tumorspheres revealed increased invasive phenotype, expression of self-renewal related genes and epithelial-mesenchymal transition. Development of therapeutic resistant 3D pancreatic cancer tumorspheres allowed for the characterisation of the CSC-associated phenotype in response to chemotherapy which more accurately reflects the cellular architecture and heterogeneity *in vivo*.

Poster Presentation (P-42)

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CelVivo bioreactor system for tissue mimicking 3D cell culture – "StressFree3D"

The CelVivo next generation 3D omnigravital spheroid cell culture system creates an environment which promotes growth and maintenance of large 3D tissue mimetic structures, whether they are spheroids, organoids, or other aggregates. In this environment, liver spheroids remain in a stable dynamic equilibrium and exhibit human *in vivo* physiological performance for at least 24 days (i.e. up to at least 42 days of culture). During this time the spheroids exhibit a stable transcriptome profile. Several principles promote this recovery process. We have found evidence that one of these basic principles is hypoxia, which is a natural consequence of growing cells in tissue like structures. In vitro hypoxia drives a partial metabolic reprogramming to aerobic glycolysis and an increased anabolic synthesis. Those adaptative changes in cellular metabolism lead to cytoskeletal reconstruction, histone modification, and recovery of the physiological phenotype.

These changes are coordinated by mTOR/Akt, p53 and non-canonical Wnt signaling pathways. Partial metabolic reprogramming to aerobic glycolysis, originally described by Warburg, is independent of the cell's rate of proliferation, but is interwoven with the cells abilities to execute advanced functionality needed for replicating the tissues physiological performance.

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