"IN VITRO 3-D TOTAL GUIDANCE AND FITNESS"

PROCEEDINGS OF THE CELLFIT WORKSHOP 2018

AND

"INTERCELLULAR EPIGENOMICS"

SEARMET AND TRANSGENO JOINT WORKSHOP 2018

TARTU, ESTONIA 10 – 11 APRIL , 2018

EDITORS

ÜLLE JAAKMA, STEFAN PRZYBORSKI, MARKUS RIMANN,

TIZIANA BREVINI, VICTORIA JAMES, ALIREZA FAZELI

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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.

WELCOME FROM THE CHAIRMAN

Welcome to the first Workshop of our COST Action CA16119, that will take place in Tartu, the *City of Good Thoughts* and the second largest city of the country. Tartu is often considered the intellectual centre of Estonia, since it is home to the nation's oldest and most renowned universities; the University of Tartu and the Estonian University of Life Sciences, which both together will host our workshop.

We will enjoy an exciting scientific day, with outstanding speakers from many different COST countries, to discuss about techniques such as 3D cell culture and bioprinting that allow the generation of biologically relevant tissue models for different applications. I do hope this first workshop will encourage many collaborations in the field, and will further implement synergies and networking within the Action participants.

I would like to thank Ülle Jaakma and all the other members of the local organizing committee for the fantastic work they have done in organizing this event. Also many thanks to our WG1 Leaders for their input in composing the program and to Shaghayegh Basatvat for managing the technical aspects.

Aitäh külalislahkuse eest!

Prof Tiziana A.L. Brevini
Chair of CellFit
April 2018

WELCOME ADDRESS FROM WORKING GROUP 1 LEADERS

Dear CellFit Members

Welcome to the first Workshop of our COST Action CA16119 that will focus on the activities of the 3-D culture technology and bioprinting Work Group. The creation of tissue-like constructs in vitro requires the development and application of new approaches. At this Workshop, we will hear from leading researchers developing some of the latest innovative technologies to enhance the structure and function of cultured cells. Attendees will learn about techniques such as 3D cell culture and bioprinting that allow the generation of biologically relevant tissue models for different applications.

We present a programme of excellent speakers from the fields of 3D cell culture and bioprinting and look forward to fruitful discussions, networking, and the development of collaborations to make new advances in this exciting field of research.

Kind regards,

Prof Stefan Przyborski (WG 1 leader)

Dr Markus Rimann (WG1 vice leader)

WELCOME ADDRESS FROM THE LOCAL ORGANISER

Dear CellFit members,

Welcome to Tartu, the university town and intellectual centre of Estonia. We believe in the very special “Spirit of Tartu“ that inspires not only citizens but also our guests. The spirit of Tartu is embedded in the Old Town cobblestones, carved into the historic buildings and appears often around the students, university buildings and numerous scientific or cultural events.

Tartu has been a university town and a city for young people for a long time. The University of Tartu – Estonia’s largest university – was established in 1632. The Veterinary Institute as a predecessor of the Estonian University of Life Sciences, was founded in 1848.

The city has been renowned as a centre of expertise in medical and veterinary research, and health technologies. We are excited about the opportunity to host the workshop of the COST Action CA16119 CellFit on bioprinting and 3D cell culture. This event will bring us new knowledge on how to create synergy between bio-engineering and clinical research. I believe that this event will strengthen the collaboration between the researchers of different countries and inspires young scientists.

Tere tulemast! Welcome!

Ülle Jaakma

Vice-Rector of Research

Estonian University of Life Sciences

WELCOME ADDRESS FROM THE INTERCELLULAR EPIGENOMICS SCIENTIFIC COMMITTEE

Dear participants of the “Intercellular Epigenomics” Meeting

Please accept our very warm welcome and thanks for participating in this meeting.

As you all know, intercellular communication is a general term used in physiology and medicine to characterize different means of communication between living cells. Cells need to communicate with each other to survive and accomplish their tasks. They use different means of communication and utilize different chemicals and factors. A comprehensive collection of literature exists that describe in detail these compounds and the role that each may play in mediating intercellular communication.

In recent years, a new paradigm has come to the fore, pointing to the role that the transfer of nucleic acids, and in particular different forms of non-coding RNA, may play in the concept of cell-cell communication. There are several examples that cells transfer/exchange genetic messages with each other in the context of both maintaining health and disease pathogenesis.

For this meeting, we aimed to gather lectures/experts to illustrate different examples of cell-to-cell communication that potentially are involved with the transfer of genomic materials from one cell to another cell, with effects on cellular function and the health of an individual. We thought the best approach to understand how this phenomenon is regulated and what the current gaps in knowledge are, was to gather people from different fields studying this subject under one roof together and hearing their experiences. We are very grateful to all the lecturers for accepting our invitation to come to Tartu. We look forward to a very fruitful day with lots insightful and lively discussion.

In organising this meeting we took advantage of the help and advice of many colleagues and friends. In particular, we would like to mention Olivika Zeiger, Marie Reeman, Shaghayegh Basatvat and Publicon crew for their help and professional service that made this meeting a reality.

Scientific organizing committee of “Intercellular Epigenomics” meeting

Alireza Fazeli, Victoria James and Ulle Jaakma

PROGRAMME ON APRIL 10TH , 2018 (ARRANGED BY CELLFIT COST ACTION 16119)

07:00-08:30 Breakfast for those accommodated at the VSpa Hotel

08:30 Ordered buses leaving from the VSpa Hotel to the University of Life Sciences

08:00-09:00 The registration desk is open at the University of Life Sciences

09:00-09:15 (15:00) Welcome address and introduction (Prof. Stefan Przyborski, Durham University, United Kingdom)

Theme 1: 3D Cell Culture Models

09:15-09:55 (40:00) **Clinostat based spheroid cultures - recovery of physiological functions through self-assembly of mimetic tissue structures.** (Dr. Krzysztof Wrzesinski, CelVivo IVS, Denmark)

09:55-10:10 (15:00) **3D strategies for improving hnMSC derived hepatocyte-like cells (HLCs) for in vitro toxicology applications.** (Dr. Joana P. Miranda, University of Lisbon, Portugal) *selected from abstracts*

10:10-10:25 (15:00) **The application of 3D cell model for genotoxicity testing.** (Prof. Bojana Zegura, National Institute of Biology, Slovenia) *selected from abstracts*

10:25-11:00 (35:00) Coffee break and Poster presentation

11:00-11:40 (40:00) **The extracellular environment of cells and how we can get them to build it in vitro.** (Prof. Michael Raghunath, Zurich University of Applied Sciences, Switzerland)

11:40-11:55 (15:00) **Animal-derived versus recombinant photo-cross linkable hydrogels for Adipose tissue engineering** (Liesbeth Tytgat, University of Vrije, Belgium) *Selected from abstracts*

11:55-14:15 (140:00) Lunch

Theme 2: Bioprinting and Biofabrication

14:15-14:45 (30:00) COST Rules of Travel reimbursement

14:45-15:25 (40:00) **Challenges and Promises of Cartilage bioprinting.** (Prof. Marcy Zenobi-Wong, ETH Zurich, Switzerland)

15:25-16:05 (40:00) **Engineering skeletal muscle tissue with innovative 3D bioprinting approaches.** (Dr. Marco Costantini, Campus Bio-Medico University of Rome, Italy)

16:05-16:45 (40:00) Coffee break

16:45-17:25 (40:00) **Improving in vitro models of cancer using 3D bioprinting.** (Prof. Nicholas R. Leslie, Heriot Watt University, United Kingdom)

17:25-17:45 (20:00) Wrap up, discussion

19:30-22:00 Gala Dinner at Joyce Restaurant

PROGRAMME ON APRIL 11TH, 2018 (OPERATED BY SEARMET AND TRANSGENO)

07:00-08:00 Breakfast at the VSpa Hotel (afterwards transfer to University of Life Sciences)

08:00-09:00 Registration desk open (University of Life Sciences)

09:00-09:15 (15:00) Welcome address and introduction (Prof. Alireza Fazeli and Prof. Ülle Jaakma)

Theme 3: Intracellular Epigenomics

09:15-09:55 (40:00) **Role of exosomal microRNAs in the biology of the Tumor Microenvironment** (Dr Muller Fabbri, University of Southern California, USA)

09:55-10:35 (40:00) **The message of a heart beat: cardiomyocyte communication in apical hypertrophic cardiomyopathy** (*Dr James Smith, University of Nottingham, UK*)

10:35-11:00 (25:00) Coffee break and Poster presentation

11:00-11:40 (40:00) **The role of small nucleolar RNAs in osteoarthritis.** (Dr. Mandy Peffers, University of Liverpool, UK)

11:40-12:00 (20:00) **Effect of maternal Insulin-dependent diabetes and hormonal stimuli on embryonic microRNA expression.** (Anne Navarrete Santos, Martin Luther University, Germany) selected talk from abstracts

12:00-12:20 (20:00) **Identification of Extracellular vesicle's RNA profile, during trophoblast and Endometrial cellular communication.** (Masoumeh Eshaghi, University of Tartu, Estonia) selected talk from abstracts

12:20-14:00 (100:00) Lunch

14:00-14:40 (40:00) **Extracellular Vesicle Communication in Cancer and other complex diseases.** (Dr Vicky James, University of Nottingham, UK)

14:40-15:20 (40:00) **Extracellular vesicles-associated RNA-protein complexes as carriers of epigenetic information** Prof. Italia Di Liegro, University of Palermo, Italy.

15:20-15:40 (20:00) Coffee break

15:40-16:20 (40:00) **Extracellular vesicles in the host-parasite communication.** (Prof. Antonio Marcilla, University of Valencia, Spain)

16:20-17:00 (40:00) **Extracellular vesicles released from the commensal yeast *Malassezia sympodialis* – host-microbe interactions** (Prof. Annika Scheynius, Karolinska Institutet, Sweden)

17:00-17:20 (20:00) Wrap up, discussion

19:30-22:00 Conference dinner

ABSTRACTS OF PRESENTATIONS

Clinostat based spheroid cultures - recovery of physiological functions through self-assembly of mimetic tissue structures.

Krzysztof Wrzesinski [a], Stephen J. Fey [a], Carlemi Calitz [b], Adelina Rogowska-Wrzesinska [c],
[a] CelVivo IVS, Odense, Denmark; [b] North-West University, Potchefstroom, South Africa;
[c] University of Southern Denmark, Odense, Denmark

Cells grown as active 3D spheroid/ organoid cultures have physiological performances that mimic that seen in human tissues better than cells grown in 2D culture. Cells grown in 2D or in 3D represent two 'extremes' of cellular programming (the cultural divide). At one extreme is exponential growth with diminished functionality (as seen in wound healing or cancer, and experimentally in traditional 2D cultures) and at the other extreme is a dynamic equilibrium with very slowly proliferating cells with a highly specialized functionality (as seen in tissues and experimentally as cells grown as active 3D spheroids) fig 1. We have shown that the hepatocellular carcinoma cells HepG2/C3A grown as active 3D spheroid cultures for periods longer than 18 days have physiological performances that mimic that seen in human tissues better than identical cells grown in 2D culture.

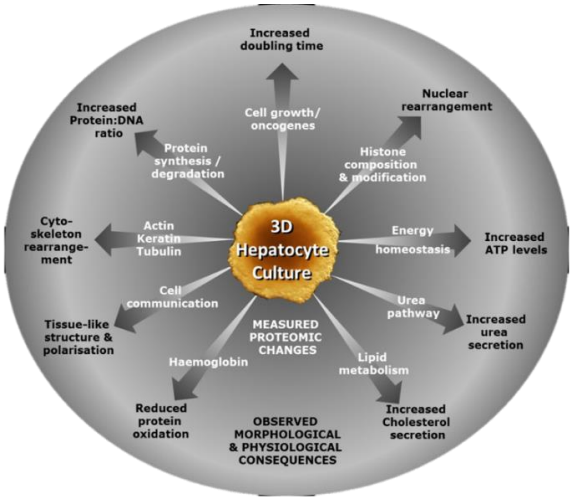


Fig. 1 Relationship of the changes in the proteome following its adaptation from 2D to 3D culture with structural and physiological properties.

Proteome and cellular architecture at these two extremes are dramatically different. Metabolically, glycolysis, fatty acid metabolism and the pentose phosphate cycle are increased while Krebs cycle and oxidative phosphorylation is unchanged. Enzymes involved in cholesterol and urea synthesis are increased underpinning the attainment of cholesterol and urea

production rates seen in vivo. DNA repair enzymes are increased. There are numerous coherent changes in transcription, splicing, translation, protein transport, folding and degradation. The amount of individual proteins within complexes is shown to be highly coordinated. Typically, subunits which initiate a particular function are present in increased amounts compared to other subunits of the same complex.

Conclusion: 3D spheroids offer a window into in vivo physiology!

REF: K. Wrzesinski and S.J. Fey. 2018. Metabolic reprogramming and the recovery of physiological functionality in 3D cultures in micro-bioreactors. Bioengineering *in press.*

3D strategies for improving hnMSC derived hepatocyte-like cells (HLCs) for in vitro toxicology applications

Joana P Miranda, iMed.ULisboa/Faculty of Pharmacy, Universidade de Lisboa Lisboa; Madalena Cipriano, iMed.ULisboa/Faculty of Pharmacy, Universidade de Lisboa; Nora Freyer, iMed.ULisboa/Faculty of Pharmacy, Universidade de Lisboa; Knoespel F., Bioreactor Group, Berlin Brandenburg Center for Regenerative Therapies (BCRT), Charité Universitätsmedizin Berlin, Berlin, Germany; Santos J.M., CECA/ICETA, University of Oporto, Oporto, Portugal; Oliveira N.G., Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa. Lisbon, Portugal; Zeilinger K. Bioreactor Group, Berlin Brandenburg Center for Regenerative Therapies (BCRT), Charité Universitätsmedizin Berlin, Berlin, Germany

jmiranda@ff.ul.pt

Human-relevant data on hepatic drug metabolism and toxicity can only be generated with cell systems that exhibit all liver-specific functions. We have been focusing our studies on deriving functional hepatocyte-like cells (HLCs) from human neonatal mesenchymal stem cells (hnMSCs) by optimizing the differentiation procedure, namely with exposure to epigenetic markers, and by resorting to more physiological-like 3D-models. Global transcriptional analyses of the HLCs at day 34 showed a partial hepatic differentiation degree revealing shared expression of gene groups between HLCs and hpHeps (human primary hepatocytes). In addition, bioinformatics analysis of gene expression data placed HLCs between the HepG2 and hpHeps and distant from hnMSCs. The enhanced hepatic differentiation observed was supported by the presence of the hepatic drug transporters OATP-C and MRP-2 and gene expression of the hepatic markers CK18, TAT, AFP, ALB, HNF4A and CEBPA; and by their ability to display stable UGTs, EROD, ECOD, CYP1A1, CYP2C9 and CYP3A4-dependent activities. In addition, HLCs differentiation was attempted using self-assembled spheroids or multi-compartment membrane bioreactor models. Overall, the 3D-systems improved the liver

specific phenotype, although the differences between phenotypic impacts promoted by each model are enough to entail that optimal culture systems should be selected depending on the scientific applications. Our findings suggest a role of the epigenetic modifiers in hepatic differentiation and maturation and reinforce the importance of the cell culture environment as a key factor for the maintenance of the hepatocyte-like phenotype. Finally, this work supports the potential of hnMSC-derived HLCs as an alternative cell type for toxicological studies.

The application of 3D cell model for genotoxicity testing

Martina Stampar, Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Vecna pot 111, 1000 Ljubljana, Slovenia; Bojana Zegura, Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Slovenia; Metka Filipic, Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Slovenia; Jana Tomc, Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Slovenia

martina.stampar@nib.si

Genetic toxicology plays an essential role within hazard identification and risk assessment during the development of novel drugs, as well as chemicals, cosmetic products, food and feed additives, pesticides, herbicides and others. Throughout the early stage of drug development, a substance's ability to damage DNA through genotoxic mechanisms must be fully investigated to enable accurate and cost-effective hazard and risk assessment. For this purpose, two-dimensional (2D) cell cultures are used in the first stage of drug development before moving to in vivo experiments. However, 2D models have several limitations, such as the lack of metabolic activity. They express low levels of phase I (activation) and phase II (detoxification) metabolic enzymes required for metabolic activation of indirect acting genotoxic compounds. Therefore, it is very important and essential to develop improved in vitro cell-based systems that can more realistically mimic the in vivo cell behaviors and provide more predictive results to in vivo conditions. The three-dimensional (3D) models have improved cell-cell and cell-matrix interactions and have preserved complex in vivo cell phenotypes. Moreover, 3D hepatic models exhibit higher levels of liver-specific functions including metabolic enzymes compared to 2D models. Therefore, the aim of our study was to develop 3D cell model from human hepatoma (HepG2) cell line and to validate the model using indirect-acting model genotoxic compound, polycyclic aromatic hydrocarbon benzo(a)pyrene (BaP) that needs metabolic activation for its genotoxic activity. In three days old spheroids non-cytotoxic concentrations of BaP (10, 20 and 40 µM) after 24 hours of exposure induced dose dependent increase in the formation of DNA strand breaks determined with the comet assay. Furthermore, the transcriptomic analyses (qPCR) revealed that HepG2 cells in 3D model express important genes

of enzymes involved in phase I (CYP1A2, CYP1A1, CYP3A4) and II (UGT1A1, SULT1A1, NAT1, NAT2) metabolism, as well as DNA damage responsive genes (P53, GADD45α, CDKN1A) at mRNA level. The results were confirmed at the protein level (Western blot). Altogether, the study showed that newly developed in vitro 3D model has improved metabolic capacity and can be used for genotoxicity assessment. Nevertheless, the 3D model has to be further validated in terms of cell division and response of genotoxic stress.

The extracellular environment of cells and how we can get them to build it in vitro

Nicole Kohli, Michael Raghunath, Zurich University of Applied Sciences (ZHAW), 8820 Wädenswil, Switzerland

The overarching principle of 3D Bioprinting is the placing of cells or cell clusters in three-dimensional space to generate cohesive tissue microarchitecture. The most popular technique is to deliver cells in so-called bioinks that contains them during the printing process and ensures shape fidelity of the printed structure. Whatever the technique, the central question in bioprinting is what happens after the printing process? Will constructs collapse, will cells remodel the pre-existing scaffold, will they die or proliferate, and will they migrate and differentiate towards a mature state of the construct. The key to this lies in the formation of an intrinsic extracellular matrix (ECM) by the cells. We have been developing macromolecular crowding (MMC) to accelerate ECM deposition by a variety of cells in monolayer culture. As bioinks essentially are hydrogels, we hypothesized that cells embedded and differentiated in hydrogels would still benefit from MMC. We embedded human bone marrow-derived mesenchymal stromal cells (MSCs) in collagen hydrogel (1mgmg/ml) and studied adipogenic differentiation and extracellular matrix deposition under MMC. As in monolayer culture, MSCs under MMC deposited more ECM, and when differentiated to adipocytes formed a collagen IV cocoon around them. Moreover, some cocoons seem to connect via ECM-bridges. These findings suggest that hydrogels indeed are amenable to MMC and that it is possible to tune deposition and microarchitecture of intrinsically produced ECM inside them. This opens up opportunities to manipulate bio-ink-based bioprinting systems, including scaffold-free approaches of biofabrication as these systems are even more dependent on a cohesive ECM.

Animal-derived versus recombinant photo-crosslinkable hydrogels for adipose tissue engineering

Liesbeth Tytgat, Vrije Universiteit Brussel, Belgium; Marica Markovic, Institute of Materials Science and Technology, Technische Universität Wien, Austria; Heidi Ottevaere, Brussels Photonics, Vrije Universiteit Brussel, Belgium; Hugo Thienpont, Brussels Photonics, Vrije Universiteit Brussel, Belgium; Aleksandr Ovsianikov, Institute of Materials Science and Technology, Technische Universität Wien, Austria; Peter Dubruel, Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry (CMaC), Ghent University, Belgium; Sandra Van Vlierberghe, Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry (CMaC), Ghent University, Belgium

ltytgat@b-phot.org

INTRODUCTION

Animal-derived gelatin-based materials are frequently used for various tissue engineering applications due to their excellent cell-interactive characteristics. However, the concerns associated with the use of animal-derived materials have emerged due to batch to batch variations and the risk of pathogen transmittance. We hypothesize that recombinant collagen I peptide (RCP) (CellnestTM, Fujifilm) could be a promising alternative to solve the issues associated with gelatin from animal origin.

EXPERIMENTAL METHODS

Gelatin B and RCP were modified using 2.5 and 1 equivalents methacrylic anhydride (MA) (GEL-MOD with a degree of substitution (DS) of 97% and RCP-MOD with a DS of 90%). The materials were characterized in depth via (HR-MAS) 1H-NMR spectroscopy, rheology, swelling and gel fraction experiments. The biocompatibility of the hydrogels was evaluated by monitoring the metabolic activity of encapsulated ASC-TERT cells. 3D scaffolds were fabricated via two-photon polymerization (2PP).

RESULTS AND DISCUSSION

RCP-MOD contained 1.6 times more photo-crosslinkable groups compared to GEL-MOD. The DSC results showed that the physical crosslinking behavior of RCP-MOD was hampered due to the higher number of double bonds. The gel fraction and HR-MAS results indicated an

efficient crosslinking for both materials. The storage modulus of RCP-MOD was comparable with GEL-MOD (6 kPa). The swelling properties of RCP-MOD was lower compared to GEL-MOD, which was anticipated since RCP-MOD has 20% more hydrophobic and 24% less hydrophilic moieties than GEL-MOD. The cell tests and literature reports indicated that RCP-MOD and GEL-MOD respectively are biocompatible. RCP-MOD and GEL-MOD could be processed via 2PP into 3D scaffolds.

CONCLUSIONS

The physico-chemical properties, the biocompatibility and the processing potential of RCP-MOD are comparable with those of GEL-MOD indicating that RCP-MOD is an attractive alternative that can be used for adipose tissue regeneration.

Challenges and Promises of Cartilage Bioprinting

Marcy Zenobi-Wong, ETH Zürich, Zürich, Switzerland

Bioprinting combines cells, biological materials and biopolymers as building blocks to fabricate tissues for use in regenerative medicine. Bioprinting's great promise is to deliver tissue replacement grafts which are personalized in terms of geometry, material properties and the patient's own cells.

First generation printing materials were made from relatively inert and soft biomaterials, limiting their performance in *in vivo* weight-bearing applications. Since cells of the body reside in an extracellular matrix (ECM) rich in biological and biophysical signaling information, there is an increasing interest in identifying key features of the native ECM which are critical to tissue development and incorporating these into the so called 'bioinks' used in bioprinting.

New generations of bioinks increase the biological activity and mechanical strength of printed tissues. Using cartilage as a model tissue, our laboratory focuses on the incorporation of biological signals which promote cell proliferation and matrix deposition via growth factor binding affinity. We also develop nanocomposite printable materials to increase strength of the constructs. Together, these approaches take us steps closer to fabricating bioprinted tissue analogues with ever-closer resemblance to native tissue properties.

Selected References:

1. Kesti, M, C. Eberhardt, Pagliccia, G, Grande, D, Boss, D, Zenobi-Wong, M*, Bioprinting complex cartilaginous structures with clinically-compliant biomaterials, Adv Funct Mat, 25: 7406-7417, 2015.

Engineering skeletal muscle tissue with innovative 3D bioprinting approaches

Marco Costantini[1], *Jan Guzowski*[1], *Wojciech Święszkowski*[2], *Cesare Gargioli*[3]

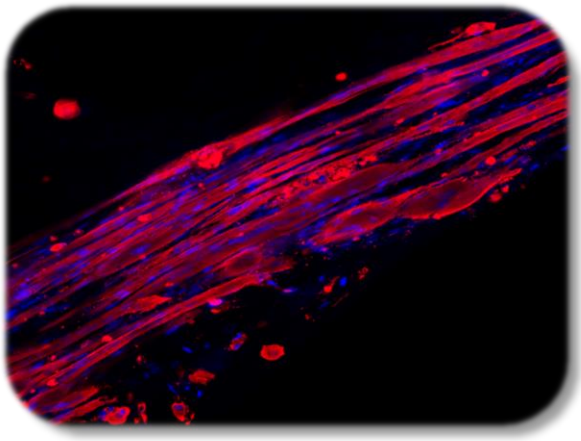
[1]*Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland.*

[2]*Warsaw University of Technology, Faculty of Materials Science and Engineering, Warsaw, Poland*

[3]*Department of Biology, University of Rome Tor Vergata, Rome, Italy*

Abstract

Within the human body, skeletal muscle (SM) tissue is responsible for a multitude of fundamental functions. All voluntary movements that characterize our lives, from a single step to a simple smile, would not be possible without this highly specialized tissue. In the last two decades, driven by its limited regenerative capacity, researchers have developed numerous approaches to restore/repair SM tissue. However, the obtained results are still far from being satisfactory.



Here, we present a new strategy for the fabrication of artificial myo-structures with functional morphologies based on an innovative 3D bioprinting approach.[1] The core of this system is a microfluidic printing head coupled with a co-axial nozzle extruding system that allows high-resolution 3D multi-cellular bioprinting of hydrogel fibers (Ø ≈ 100 μm) and high cell viability. To promote myoblast differentiation, we formulated a tailored bioink with a photocurable semi-synthetic biopolymer, namely PEGylated fibrinogen, and we encapsulate cells (C2C12 or human pericyte) in 3D scaffolds composed of unidirectionally aligned hydrogel fibers. The 3D fabricated samples were tested both *in vitro* and *in vivo* to evaluate their capability of supporting myogenesis and sarcomerogenesis. The results showed that after 21 days of culture *in vitro*,

myoblasts properly spread and fused forming highly aligned long-range multinucleated myotubes, with abundant and functional expression of myosin heavy chain and laminin. Besides, 3D biofabricated constructs when grafted *in vivo* led to a substantial improvement if compared to bulk-hydrogels (used as control) of muscle-like architectural organization with the formation of tightly-packed, highly parallel and completely striated myotube fibers.

References

[1] M. Costantini, et al., *Biomaterials* **2017**, *131*, 98.

Improving in vitro models of cancer using 3D Bioprinting

Nicholas R Leslie

Heriot Watt University, Edinburgh, UK

In-vitro brain tumour models are valuable tools both to study cancer biology and for successful pre-clinical testing of anticancer drugs. However, substantial challenges remain before we can create physiologically-relevant models which closely mimic the microenvironment of human brain tumours and reveal their response to therapies. We have developed a 3D bioprinting strategy using modified alginate matrices incorporating hyaluronan and collagen, in order to create tumour-like structures containing both glioma stem cell lines and glioma derived stromal cells. Our results to date show that the printing process can be performed with minimal effects on cell viability, even when cells are printed at high density; that cells proliferate after printing and that rapid cell-cell adhesion can be promoted with appropriate matrices. We are able to print glioma stem cells with and without stromal cells in separate layers within tumour like 3D constructs with a smallest feature size (resolution) of approximately 300μm. This capability to spatially position cell populations and other components within a 3D tumour construct allows new experimental approaches by controlling individual parameters of the tumour microenvironment and should provide novel reproducible models for preclinical drug testing.

Role of exosomal microRNAs in the biology of the Tumor Microenvironment

Muller Fabbri, MD, PhD, Children's Hospital Los Angeles- University of Southern California, Los Angeles, CA, USA

Extracellular vesicles (EVs) mediate inter-cellular communication among different cell populations of the Tumor Microenvironment. Exosomes are a subtype of EVs that carries a cargo of different types of functional macromolecules, including microRNAs (miRNAs) and other coding and non-coding RNAs. We have identified a new paracrine function of exosomal miRNAs within the Tumor Microenvironment. Cancer cells release specific miRNAs within exosomes, and this miRNAs are successfully uptaken by surrounding immune cells. We describe a "hormone-like" mechanism of miRNA binding to Toll-like receptor 8 in immune cells, triggering a pro-tumoral inflammatory response responsible for increased tumor growth and resistance to chemotherapy. We will also discuss how exosomal miRNAs derived from immune cells can elicit anti-tumoral effects and how these discoveries can be exploited for the identification and development of new anti-cancer therapies.

The message of a heart beat: cardiomyocyte communication in apical hypertrophic cardiomyopathy

James Smith, Victoria James and Chris Denning

University of Nottingham, Nottingham, United Kingdom

Hypertrophic cardiomyopathy (HCM) is a thickening of the ventricles that can lead to devastating conditions such as heart failure and sudden cardiac death. HCM is linked to mutations in genes encoding cardiac sarcomeric proteins, such as *ACTC1* encoding for actin. Despite extensive study the mechanisms mediating many of the associated clinical manifestations, such as interstitial fibrosis, remain unknown and human models are required. To address this, hiPSC lines were generated from heart patients with a HCM associated *ACTC1* mutation (E99K). Isogenic controls were created for each line by correcting the mutation using CRISPR/Cas9 gene editing technology. Isogenic pairs were differentiated to cardiomyocytes and subjected to electrical stimulation to capture the cells at a stress-inducing 3Hz contraction frequency. Following 24 hours pacing, conditioned medium was collected and exosomes isolated and characterised. The pattern of release (size and quantity) of exosomes produced by diseased cardiomyocytes was significantly altered compared to isogenic control lines. Stressed conditioned medium, containing exosomes, was shown to activate fibroblasts into a more secretory active myofibroblast state. We hypothesise that E99K mutant cardiomyocytes modify the release and content of their extracellular vesicles, and are investigating whether this causes activation of neighbouring cells to result in unexplained disease phenotypes such as fibrosis.

Small nucleolar RNAs in osteoarthritis

Mandy Peffers, University of Liverpool, Liverpool, UK

Small nucleolar RNAs (snoRNAs) are non-coding RNAs of 50-150 nucleotides long with a main task in post-transcriptional modification of ribosomal RNAs (rRNAs). A small subset of snoRNAs (eg U3), function in endoribonucleolytic processing of 47S pre-rRNA. We are currently undertaking a number of targeted and discovery studies to investigate the relevance of snoRNAs for osteoarthritis (OA) progression, cartilage ageing and OA biomarker discovery.

General chondrocyte molecular biology methods were used to isolate, culture and analyse chondrocytes, cartilage and serum/synovial fluid. Transcriptomics was performed using micro-array and RNAseq. Intervention with snoRNA expressions was performed by transfecting anti-sense oligonucleotides.

Ageing and OA development led to alterations of specific snoRNA expression in whole mouse joints. Micro-array expression profiling of young, old-protected and old-OA human cartilage demonstrated age-specific and OA-specific expression of snoRNAs. Indeed we have evidence that alterations in U3 snoRNA expression leads to OA-like changes in the chondrocyte phenotype and that U3 can be influenced by morphogens and cytokines. Also knock-down of OA-specific snoRNAs induces changes in chondrocyte differentiation status and rRNA levels. Our biomarker studies have identified ageing- and OA-specific expression of snoRNAs. We are now exploring the snoRNA content of healthy and OA synovial fluid to identify snoRNA biomarkers for early OA development and investigate whether synovial fluid-mediated inter-tissue communication within the joint involves snoRNAs.

Our data show for the first time that specific snoRNAs are differentially expressed in ageing- and OA cartilage. SnoRNAs provide an unexplored class of non-coding RNAs relevant for chondrocyte biology.

Effects of maternal insulin-dependent diabetes and hormonal stimuli on embryonic microRNA expression

Anne Navarrete Santos, Martin Luther University Halle-Wittenberg, Halle/ Germany; Mareike S .Pendzialek, Department of Anatomy and Cell Biology, Martin Luther University Faculty of Medicine, Halle (Saale), Germany; Katarzyna J. Grybel, Department of Anatomy and Cell Biology, Martin Luther University Faculty of Medicine, Halle (Saale), Germany; Jacqueline Gürke, Department of Anatomy and Cell Biology, Martin Luther University Faculty of Medicine, Halle (Saale), Germany; Maria Schindler, Department of Anatomy and Cell Biology, Martin Luther University Faculty of Medicine, Halle (Saale), Germany; Tom Seeling, Department of Anatomy and Cell Biology, Martin Luther University Faculty of Medicine, Halle (Saale), Germany; Fischer Bernd Department of Anatomy and Cell Biology, Martin Luther University Faculty of Medicine, Halle (Saale), Germany
a.navarrete-santos@medizin.uni-halle.de

Placenta-specific microRNAs (miRs) represent a new class of biological markers for prenatal diagnosis as they reflect physiological and pathological placental conditions. Placenta development starts as early as during blastocyst development with embryoblast and trophoblast differentiation. We hypothesise that specific trophoblast miRs could be a useful tool for the identification of diabetes-related placental dysfunction. Therefore, we have analysed the influence of a maternal type 1 diabetes mellitus on miR expression in vivo in maternal blood plasma and endometrium as well as in the preimplantation embryo, using the rabbit as experimental model. Further, in in vitro embryo culture we examined direct effects of insulin, glucose, IGF2 and LIF on embryonic miR expression.
Maternal diabetes led to a downregulation of miR-27b, -141, -191 and -222 in maternal plasma and endometrium and in embryonic samples (embryoblast and trophoblast cells). These miRs were also present in the blastocyst cavity fluid, but were not affected by maternal diabetes. Furthermore, we detected an altered mRNA expression of miR target genes in blastocysts developed under diabetic conditions. In in vitro experiments LIF, insulin, IGF2 and glucose

were capable to stimulate embryonic miR-27b, -141, -191 and -222. In vitro data indicate that changes in maternal hormones and metabolites regulate embryonic miR expression with potentially consequences for blastocyst development and implantation. Furthermore, the altered miR expression in both embryonic compartments and in maternal plasma during diabetic pregnancy demonstrates that miRs could serve as biological markers as early as during the preimplantation period.

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Identification of extracellular vesicle's RNA profile, during trophoblast and endometrial cells communication

Masoumeh Eshaghi Masoumeh, Tartu university, Estonia; Alireza Fazeli, Institute of Biomedicine and Translational Medicine, Department of Pathophysiology, University of Tartu, Estonia; Annika Häling, Institute of Biomedicine and Translational Medicine (Role: Co-Author); Freddy Lattekivi - Institute of Biomedicine and Translational Medicine, Department of Pathophysiology, University of Tartu, Estonia (Role: Co-Author); Stoyan Tankov - Postdoctoral researcher, Institute of Biomedicine and Translational Medicine, Department of Pathophysiology, University of Tartu, Estonia (Role: Co-Author); Victoria James - School of Veterinary Medicine and Science, University of Nottingham (Role: Co-Author); Tamer Nafee - Academic Unit of Reproductive and Developmental Medicine, Department of Oncology and Metabolism, The Medical School (Role: Co-Author); Sulev Koks - Institute of Biomedicine and Translational Medicine, Department of Pathophysiology, University of Tartu, Tartu, Estonia (Role: Co-Author)

masoumeh.eshaghi@ut.ee

Extracellular vesicles (EV) could be a mean for maternal and embryonic cell lines communication. This study has aimed to identify the RNA profile of extracellular vesicles that exchanged between trophoblast and endometrial cells during communication. The 5-Ethynyl uridine (EU) labeled trophoblast spheroids were cultivated with an endometrial cell line in a non-contact co-culture system. The trophoblast EU-labeled RNA was tracked and captured in endometrial cells. The transferred labeled RNA, was affinity-precipitated and purified using biotin-azide click chemistry. Total RNA-sequencing was conducted with synthesized cDNA from captured labeled and non-EU labeled RNA (background) (n=4). Differential expression analysis of RNA-seq data was performed using edgeR and limma packages to identify the transferred transcripts using differential enrichment as a proxy. The Integrative Genomics Viewer was used to validate the coverage of differentially enriched transcripts. The results were confirmed by Quantitative PCR (qPCR). To establish the route of candidate RNA transfer, EVs were isolated from co-culture media using size exclusion chromatography. Total RNA was extracted from EVs, EU-labeled RNA was affinity-

precipitated and the absolute copy number of putatively transferred RNA sequences was quantified.

Differential enrichment analysis demonstrated that the majority of putatively transferred transcripts were non-coding RNAs derived from the mir99alet7c cluster (Chromosome 21: LINC00478). The presence of non-coding sequences from this chromosomal region in the RNA extracted from EVs was confirmed by qPCR. This suggest that these sequences are carried by throphoblast EVs.

In this study we showed that bioortogonal RNA labeling chemistry can be used for the deciphering trophoblast-endometrial communications. These are the initial steps towards decoding the earliest stages of the mother-offspring language/cross talk.

Communication of prostate cancer cells with bone cells via extracellular vesicle RNA; a potential mechanism of metastasis

Probert C[1], Dottorini, T[1,2], Speakman A[1], Hunt S[3], Nafee T[4], Fazeli A[4,5], Wood, S[6], Brown JE[6], James V[1]*

[1] School of Veterinary Medicine and Science, University of Nottingham, Nottingham, LE12 5RD

[2] Advanced Data Analysis Centre, University of Nottingham, Nottingham, LE12 5RD

[3] School of Clinical Dentistry, University of Sheffield, Claremont Crescent, Sheffield, S10 2TA

[4]Academic Unit of Reproductive and Developmental Medicine, University of Sheffield, Jessop Wing, Tree Root Walk, Sheffield S10 2SF

[5]Institute of Biomedicine and Translational Medicine, Department of Pathophysiology, University of Tartu, Tartu, Estonia

[6] Department of Oncology and Metabolism, University of Sheffield, Western Park Hospital, Whitham Road, Sheffield, S10 2SJ

The role of extracellular vesicles (EVs) as vehicles for cell-to-cell communication between a tumour and its environment is a relatively new concept. However, the hypothesis that EVs may play a critical role in co-opting tissues by tumours to generate a metastatic niche is generating significant interest. Using prostate cancer as a model, we demonstrated the potential role of EVs as a mechanism of mediating prostate cancer to bone metastasis, and specifically the contribution of the EV-RNA cargo as a means of communication between prostate cancer cells and cells of the bone stroma.

We demonstrate the effect of prostate cancer cell EVs on osteoblast proliferation and apoptosis, and how exposing osteoblasts to prostate cancer EVs creates a significantly more supportive growth environment for prostate cancer cells when grown in co-culture ($p<0.005$). Characterisation of the RNA cargo of EVs produced by the bone metastatic prostate cancer cell line PC3, highlights that the EV-RNA cargo is significantly enriched in genes relating to cell surface signalling, cell-cell interaction, and protein translation ($p<0.01$). Using techniques to track RNA originating from the prostate cancer cell, we demonstrate the delivery of a set of prostate cancer originating RNAs to osteoblast via prostate cancer EVs and show the effect on osteoblast endogenous transcript abundance. Together, this work advances our understanding

of EV function in cancer and beyond by highlighting the contribution of the RNA element of the delivered cargo.

Extracellular vesicles-associated RNA-protein complexes as carriers of epigenetic information

Carlo Maria Di Liegro[1], Gabriella Schiera[1], and Italia Di Liegro[2]

[1]Department of Biological Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), and [2]Department of Experimental Biomedicine and Clinical Neurosciences (BIONEC), University of Palermo, Palermo I-90127, Italy; italia.diliegro@unipa.it

Background: Cancer cells are much more active than normal cells in producing different kinds of extracellular vesicles (EVs), through which they transfer to the surroundings transforming nucleic acids and proteins [1]. We previously found that EVs released from G26/24 oligodendroglioma cells can transfer, via EVs, various proteins, among which pro-apoptotic factors [2], extracellular matrix remodeling proteases (such as ADAMTS) [3], and the H1.0 histone protein [4]. Similarly, A375 melanoma cells release EVs containing a sumoylated form of H1.0, as well as H1.0 RNA [5].

Aim: To investigate the possibility that EVs contain specific RNA-protein complexes with the potential to induce epigenetic modifications in recipient cells.

Methods: EVs were purified from A375 melanoma cells. Protein extracts from both cells and EVs were used for T1 RNase-protection assays, using as target the H1.0 RNA. In a second step, we used affinity chromatography on a biotinylated H1.0 RNA to fish H1.0 RNA-binding proteins, to be finally identified by mass spectrometry.

Results: Three main complexes were evidenced, the most abundant of which had an apparent molecular mass of about 65 kDa. In the second step, we identified myelin expression factor-2 (MYEF2) as a major H1.0 mRNA-binding protein in EVs released from melanoma cells [5].

Conclusion: MYEF2 was already known as a transcription factor with putative RNA-binding domains. Our demonstration of its actual ability to bind RNA rises the interesting possibility that RNA can function as a carrier of factors that, once in the receiving cells, can change their transcriptional activity.

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Extracellular vesicles in the host-parasite communication

Marcilla, A., Galiano, A., Sánchez, C.M., Cifre, S., Cantalapiedra, F., Trelis, M., Bernal, D.
Universitat de València, Burjassot (Valencia), Spain

Parasitic diseases are a major public health issue, with billions of people infected mainly in poor regions, and some of them are considered as neglected tropical diseases (1). Parasites have evolved complex life cycles involving different hosts provoking a wide range of clinical outcomes from asymptomatic to severe infections, including death (2). The host-parasite interaction require refined pathogen-specific intercellular communications, where extracellular vesicles seem to play a pivotal role (3, 4). Extracellular vesicles are well recognized as mediators of intercellular communications in prokaryotes and eukaryotes, transferring functional information to recipient cells that signal normal and pathophysiological processes (5). Since these vesicles contain pathogen-derived molecules, they represent novel approaches for identifying biomarkers of disease, as well as new antigens for vaccination (5).

We will discuss our experience in the isolation and characterization of parasite extracellular vesicles, as well as their possible applications in controlling parasitic diseases, as new diagnostic tools (biomarkers) and their usefulness in vaccination assays. Along with parasitic diseases, we will also address their usefulness in treating autoimmune diseases.

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Extracellular vesicles released from the commensal yeast *Malassezia sympodialis* – host-microbe interactions

Annika Scheynius, Department of Clinical Science and Education, Södersjukhuset, and SciLifeLab, Karolinska Institutet, Stockholm, Sweden

Malassezia is a dominant commensal fungi in the human skin mycobiome but is also associated with common skin disorders including atopic eczema (AE). More than 50 % of AE-patients have specific IgE and T-cell reactivity towards *Malassezia sympodialis*, which is one of the most frequently isolated species from both AE patients and healthy individuals. We have found that *M. sympodialis* releases extracellular nanovesicles, designated MalaEx, which are carriers of allergens and can induce inflammatory cytokine responses. To further elucidate *M. sympodialis* host-microbe interactions we assessed whether small RNAs are present in MalaEx and could identify a set of reads in a length range of 16 to 22 nucleotides. Interestingly, these RNA features appear to have an RNAi-independent route for biogenesis. We further compared the protein content of MalaEx with that of the parental yeast cells. iTRAQ based quantitative proteomics identified in total 2439 proteins in all replicates of which 110 were enriched in MalaEx compared to the yeast cells. Among the MalaEx enriched proteins were two of the major *M. sympodialis* allergens, Mala s 1 and s 7. Functional experiments indicated an active binding and internalization of MalaEx into human keratinocytes and monocytes, and MalaEx were found in close proximity of the nuclei using super-resolution fluorescence 3D-SIM imaging. Our results provide new insights into host-microbe interactions, supporting that MalaEx may have a role in the sensitization and maintenance of inflammation in AE by containing enriched amounts of allergens and with their ability to interact with skin cells.

3D gelatin scaffolds

Martin Järvekülg, University of Tartu, Riga Technical University, Tartu/Estonia; Paula Reemann, University of Tartu; Viljar Jaks, University of Tartu; Aigar Ottas, University of Tartu; Külli Kingo, University of Tartu; Karlis Gross, Riga Technical University

martin.jarvekylg@ut.ee

Gelatin is produced by hydrolysis from collagen, the most prominent extracellular matrix protein. Therefore, gelatin can be presents chemical composition that and biochemical properties that are well suited for designing artificial extracellular matrices (ECM). We have developed a strategy where glucose is added to achieve additional crosslinking during thermal treatment, resulting a biocompatible material that preserves its nanoscale morphology in wet state. It has been demonstrated in recent studies that the 3D surroundings its local mechanical properties also play a critical part in directing cell decisions. Hereby we report the results of several material design approaches to 3D arrangements of fibrous glucose-crosslinked gelatin. Electrospinning was used to produce gelatin nanofibers. Obtained material was processed into a suspension of short fibers, which was thereafter used to prepare fibrous sponges. Other porous gelatin materials were prepared by immersion precipitation and an alternative spinning method. Obtained scaffolds were subject to cell culture studies using human dermal fibroblasts and Hepg2, mass spectroscopy, fluorescence microscopy, SEM and mechanical characterization. While strong cell-material interactions were observed on all gelatin scaffolds, superior cell penetration into deper layers of the material. Ongoing work is dedicated to developing a ECM platform for developing tissue equivalents and disease models

Peroxisome proliferator-activated receptor (PPAR) ligands regulate nuclear factor kappa-B (NF-κB) gene and protein expression in the porcine endometrium on days 14-16 of the estrous cycle and pregnancy

Zuzanna Kunicka, University of Warmia and Mazury in Olsztyn, Olsztyn / Poland; Iwona Bogacka, University of Warmia and Mazury in Olsztyn; Anna Szydłowska, University of Warmia and Mazury in Olsztyn; Aleksandra Kurzyńska, University of Warmia and Mazury in Olsztyn

zuzanna.kunicka@uwm.edu.pl

Nuclear factor kappa-B (NF-κB) is a transcriptional factor involved in the modulation of immune response, cell proliferation and apoptosis in various cells types, including those of the endometrium. It controls the synthesis of proinflammatory cytokines regulating reproductive processes. The present study aimed to evaluate the effect of peroxisome proliferator-activated receptor (PPAR) ligands on NF-κB gene and protein expression in the endometrium of sows, depending on their reproductive status.

Porcine endometrial slices, collected at days 14-16 of the estrous cycle (late-luteal phase; n=5) or pregnancy (beginning of implantation; n=5), were incubated in vitro for 6 h in the presence of PPARα (agonist WY 14643 and antagonist MK-886), PPARβ (agonist L-165,041 and antagonist GW9662) or PPARγ (agonists: 15d-prostaglandin J2 (PGJ2) or rosiglitazone and antagonist T0070907) ligands. The mRNA and protein levels in the tissue were determined by Real Time PCR and Western Blot, respectively. The effect of the treatment was determined by one-way analysis of variance followed by the Duncan post hoc test.

During the estrous cycle, the agonists of three PPAR isoforms (alfa, beta and gamma) diminished NF-κB mRNA expression in the porcine endometrium. In addition, the inhibitory effect of PPARβ and PPARγ (but not PPARα) agonists on NF-κB protein level was noted. During pregnancy, the agonists of PPARα and PPARβ (but not PPARγ) reduced the expression of NF-κB mRNA, whereas the tested compounds did not affect the protein level.

The obtained results imply the involvement of PPARs in the expression of NF-κB in the porcine endometrium. They indicate a diverse receptivity of the tissue to the PPAR ligands, depending

on the reproductive stage of sows. The impact of the ligands on NF-κB gene and protein expression in the endometrium is relevant during the estrous cycle what may suggest that PPARs, as anti-inflammatory factors, modulate the immune system during luteolysis.

Chemokines could promote porcine endometrial epithelial cells remodelling

Aneta Andronowska; Institute of Animal Reproduction and Food Research Polish Academy of Sciences; Olsztyn, Poland; Krzysztof Witek, Institute of Animal Reproduction and Food Research Polish Academy of Sciences in Olsztyn; Aleksandra Złotkowska, Institute of Animal Reproduction and Food Research Polish Academy of Sciences in Olsztyn

a.andronowska@pan.olsztyn.pl

Preparation of endometrium to embryo implantation is a crucial process controlled by many factors including great number of cytokines. Chemokines (small proteins belonging to cytokines family) are known mainly because of their role in chemoatraction of immune cells to inflammation site. Nowadays, still new important role of these proteins in different cell types is being discovered.

The aim of our study was to identify chemokine/-s which can contribute to preparation of epithelial cells for proper embryo implantation.

Endometrial epithelial cells were obtained using enzymatic digestion of tissue from mature gilts. After purification, cells were cultured to reach 70% confluence and then treated with recombinant chemokines in physiological concentration (1ng/ml). Proliferation analysis was performed using colorimetric Cell Proliferation Assay. Cells ability to adhesion was examined using Crystal Violet staining. Gene expression was measured by real-time PCR. All data were statistically analyzed with 1-way ANOVA followed by NIR Fisher test.

Analysis of gene expression disclose presence of transcript for all examined chemokines' receptors in epithelium, with the highest expression of CXCR4 (receptor for SDF1). All chemokines significantly ($p<0.001$) enhanced epithelial cell proliferation. Adhesion assay revealed only SDF1- positive effect and CCL8- negative effect on cell adhesion in comparison to control group. Expression on mRNA for Muc-1 coincide with adhesion results, indicating inhibitory effect of SDF1 on Muc-1 expression. Downregulation of Muc-1 was also observed after CXCL2 and CXCL9 stimulation.

In conclusion, our results show that some of chemokines may affect functions of epithelial cells. Especially, SDF1 with positive effect on cells proliferation and adhesion, may be the one of crucial factors contributing to appropriate embryo implantation in non-invasive type of implantation in pig.

Hormonal regulation of prostaglandin synthesis in an air-liquid interface culture of porcine oviductal epithelial cells (ALI-POEC)

Aleksandra Złotkowska; Institite of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland; Jennifer Schoen, Leibniz Institute for Farm Animal Biology, FBN Dummerstorf, Germany; Aneta Andronowska, Institite of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland; Shuai Chen, Leibniz Institute for Farm Animal Biology, FBN Dummerstorf, Germany

a.zlotkowska@pan.olsztyn.pl

Previously, we reported an air-liquid interface approach to culture oviductal epithelial cells, which recapitulated polarization and ciliogenesis of oviduct epithelia in vivo and allows long-term cultivation as well as estrous cycle simulation. Prostaglandins are known to participate in embryo development and transport; however, the hormonal regulation of their synthesis remains unclear. Thus, aim of this study was to investigate the expression of key enzymes of the prostaglandin synthesis pathway during in vitro simulation of estrous cycle stages in ALI-POEC.

We sequentially simulated diestrus and estrus stages in ALI-POEC using serum levels of estradiol (E2) and progesterone (P4) either in combination (mix group) or separately (P4 and E2 group). The control group received solvent only. Gene expression was measured by RT-qPCR, while localization of proteins was determined by immunofluorescence.
In the mix group, gene expression of mPGES-1 was significantly higher in diestrus than in estrus. In the separate simulations, P4 caused up- and E2 down- regulation of mPGES-1 expression. Expression of PGFS was increased in estrus in comparison to diestrus in all groups. No differences in PTGS2 and PGIS expression were revealed. PGIS and mPGES-1 proteins were localized in cytoplasm of all, whereas PGFS and PTGS2 were present in cytoplasm and membranes of individual cells. CBR1 protein was specifically observed in the apical parts of the epithelial cells.

In conclusion, taking advantage of the polarized ALI-POEC system, this preliminary study provided evidence for the regulation of the prostaglandin synthesis pathway by E2 and P4 in the oviduct during the estrous cycle.

Confocal imaging of 3D spheroids from mesenchymal stem cells

Milena Mourdjeva, Institute of biology and immunology of reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria; Shina Pashova, Institute of biology and immunology of reproduction, Bulgarian Academy of Sciences; Milena Kostadinova, Institute of biology and immunology of reproduction, Bulgarian Academy of Sciences; KameliyaVinketova, Snejana Kestendjieva, Desislava Abadjieva, Elena Stoyanova, Tsvetelina Oreshkova, Elena Kistanova, Milena Mourdjeva

milena_mourdjeva@abv.bg

MSCs have an intriguing property of in vitro self-assembly into three-dimensional (3D) aggregates. Recent studies have shown that MSC 3D aggregation enhances a range of biological functions, such as proliferation, potential for multilineage differentiation, secretion of therapeutic factors, immunomodulation, anti-tumour properties thus leading to improved therapeutic potential.

Adipose tissue derived MSCs cultured in hanging drops formed a loose network of grouped cells at day 1. At day 2 the numerous small aggregates shrank and formed a single compact structure. These structures were fixed and the prestained with CFSE (cytoplasm) and Hoechst (nuclei) MSCs in the spheroids were analyzed by confocal imaging. The result revealed that the cell number is inversely proportional to the size of the formation. The smallest structures were formed from 21000 cells and reached the size of 480+/- 50 µm, and the biggest spheroids contained 10000 cells and were 675+/- 30 µm in size. The confocal imaging revealed non-homogeneous structures, consisting of cells with different shape and dimensions and a lot of cavities. The cells in the spheroid surface were smaller than those in the inner part. In the course of time the spheroids grew (480 +/-50 µm at day 2; 700 +/-20 µm at day 3, and 750 +/-60 µm at day 6), became more compact and reduced the cavities by day 6. Our data showed that almost all MSCs in the spheroids are alive (Fixable Viability Dye eFluor 450) and proliferating. In the next step of our work we are going to measure the proliferation of MSC in spheroids by flowcytometry.

Regulation of the membrane progesterone receptors expression in the human placental choriocarcinoma cell line JEG-3

Karolina Krajza, Institute of Animal Reproduction and Food Research Polish Academy of Sciences, Poland; Magdalena Kowalik, Institute of Animal Reproduction and Food Research Polish Academy of Sciences; Jan Kotwica, Institute of Animal Reproduction and Food Research Polish Academy of Sciences
k.krajza@pan.olsztyn.pl

Progesterone (P4) affects cells function by genomic mechanism through nuclear P4 receptors, and via nongenomic mechanism through the membrane P4 receptors, such as: progesterone receptor membrane component (PGRMC) 1 and 2 and membrane progestin receptors (mPR) alpha (mPRα), beta (mPRβ) and gamma (mPRγ). The presence of membrane P4 receptors has also been demonstrated in human JEG-3 choriocarcinoma cells though the mechanism that regulate the expression of membrane P4 receptors are unknown. It should be emphasized the cell line JEG-3 is a reliable and used model for studying the secretory function of placental trophoblast and the participations of receptors in these processes. Therefore, the aim of study was to examine the effect of chorionic gonadotropin (hCG; 10ng/ml), P4 (10-5M), estradiol (E2; 10-9M) and 5% amniotic fluid (AF) on gene expression for: PGRMC1, PGRMC2, mPRα, β and γ in cell line JEG3 (1,2x105/ml). After 6 (n=4) and 24h (n=6), the concentration of P4 and E2 in medium and mRNA expression of studied genes were determined by an enzyme-immunoassay (EIA) and Real Time PCR method, respectively. Obtained data indicate, that P4 and AF increased concentrations of E2 in medium ($p<0.05$) after 6 and 24h. Moreover, P4 stimulated ($p<0.05$) expression of mPRγ and PGRMC2 while AF decreased ($p<0.05$) expression of mPRβ after 6h of incubation. These results suggest that steroids and amniotic fluid may regulate secretory function and expression of mPRβ, mPRγ and PGRMC2 mRNA in cell line JEG-3.

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The effect of progesterone and estradiol on membrane progesterone receptors gene expression in the bovine myometrial cells

Magdalena K. Kowalik, Institute of Animal Reproduction and Food Research Polish Academy of Sciences, Olsztyn/ Poland; Karolina Krajza, Institute of Animal Reproduction and Food Research Polish Academy of Sciences; Jan Kotwica, Institute of Animal Reproduction and Food Research Polish Academy of Sciences
m.kowalik@pan.olsztyn.pl

We found the variable expression of mRNA and protein for progesterone receptor membrane component (PGRMC) 1 and 2, PGRMC1 binding partner – SERBP1 (serpine 1 mRNA binding protein) and membrane progestin receptors (mPR) α, β and γ in bovine myometrium during the estrous cycle but the regulatory mechanism of mRNA expression of these receptors in myometrium is not clear. The aim of the present study was to examine the effect of P4 (10-7;10-6;10-5M), estradiol (E2; 10-10;10-9;10-8 M), P4 (10-6M or 10-5M) together with E2 (10-9M or 10-8M) and arachidonic acid (AA;10-5M as positive control) on gene expression for: PGRMC1, PGRMC2, SERBP1, mPRα, β and γ in the bovine myometrial cells (2.5x105/ml; n=5) from days 6-10 and 11-16 of the estrous cycle. After 6h, the concentration of PGF2α and PGE2 in medium and mRNA expression of studied genes were determined by EIA and Real Time PCR. Obtained data indicate that E2 (10-8M) stimulated ($P<0.05$) expression of PGRMC1 mRNA, and P4 (10-5M) and P4 together with E2 increased ($P<0.05$) mPRβ mRNA in the cells from 6-10 days of the estrous cycle. In the cells from 11-16 days of the estrous cycle, P4 (10-6 M) and E2 (10-10M) decreased ($P<0.05$) only SERBP1 mRNA, but P4 in the dose of 10-5M increased ($P<0.05$) the expression of this gene. These results suggest that steroids may regulate PGRMC1, SERBP1 and mPRβ mRNA expression in the bovine myometrial cells and this effect depends on the days of estrous cycle and steroids dose. Supported by Grant NCN 2012/05/B/NZ4/01810 and by Polish Academy of Sciences.

Growing artificial bone in rotating bioreactors, a model system for identifying molecular mechanisms of treatment

Maria Schröder, Department of Biomaterials, University of Oslo, Oslo/Norway; Elisabeth Riksen, Department of Biomaterials, University of Oslo; Janne E. Reseland, Department of Biomaterials, University of Oslo

maria.schroder@odont.uio.no

Rotating bioreactors on a clinostat system create an environment that allows bone cells to aggregate into 3D multicellular spheroids and form bone-tissue like structures 1,2. These 3D in vitro analogs of human bone represent a new potential model for testing of various stimuli on bone formation and remodeling 2. The active vitamin D metabolite 1,25(OH)2D has a known function in bone homeostasis. Vitamin K2 is a cofactor of gamma-carboxylase, and essential for the pathway of gamma-carboxylation of osteocalcin (OC) 3. Studies showed a stimulating effect of a combined administration of vitamin D3 and K2 on osteoblast mineralization 4,5 and bone mineral density in vivo 6,7.

In this study, the effect of vitamin K2 alone and in combination with vitamin D3 is tested in two different rotary culture systems in the 3D bone model. Results are compared to each other and to effects in a 2D culture system.

3D mineralized bone constructs were generated either in the rotary cell culture system (RCCS) (Synthecon, Inc., Houston, TX) or the BioArray Matrix drive BAM v4 (CelVivo, Blommenslyst, Denmark) with human primary osteoblasts (hOBs) and osteoclast precursor cells. Vitamin D3 and K2 (both MK-4 and MK-7), alone and in combination, were administrated. Untreated cells were used as control. After 21 days, osteospheres were characterized in terms of mineralization by Alizarin Red S staining, Alkaline Phosphatase activity and expression of collagen type I. The secretion of cytokines and bone factors to the medium was analyzed by Luminex.

In a 2D culture system, the secretion of OC from hOBs was highest by a combined treatment of vitamin K2 form MK-4 (10 µM) and vitamin D3 (10-8 M). In the 3D bone model, vitamin treatment distinctly reduced osteosphere stiffness compared to the untreated control.

Ex vivo-derived 3D bone constructs may be utilized in physiological studies of bone formation, remodeling and function.

JE Reseland is a member of Cost action CA16119 CellFit

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PARTICIPANTS

Aneta Andronowska	**Poland**	**a.androwska@pan.olsztyn.pl**
Daiva Baltriukienė	Lithuania	daiva.baltriukiene@bchi.vu.lt
Marco Costantini	Poland	marco.costantini@uniroma1.it
Italia Di Liegro	Italy	italia.diliegro@unipa.it
Keerthie Chulanga Dissanayake	Estonia	keerthiedissanayake@yahoo.com
Monika Dovenska	Macedonia	mdovenska@fvm.ukim.edu.mk
Toni Dovenski	Macedonia	dovenski@fvm.ukim.edu.mk
Yasar Murat Elcin	Turkey	elcinmurat@gmail.com
Masoumeh Es-haghi	Estonia	masoumeh.eshaghi@ut.ee
Muller Fabbri	United States of America	mfabbri@chla.usc.edu
Alireza Fazeli	Estonia	alireza.fazeli@ut.ee
Anthony Fenech	Malta	anthony.fenech@um.edu.mt
Kasun Godagedara	Estonia	kasungodakumara@gmail.com
Irina Guljavina	Estonia	irina.guljavina@gmail.com
Yongzhi Guo	Sweden	yongzhi.guo@slu.se
Mohammad Mehedi Hasan	Estonia	rajibmehedi3@gmail.com
Patrice Humblot	Sweden	patrice.humblot@slu.se
Marilin Ivask	Estonia	marilin.ivask@ut.ee
Ülle Jaakma	Estonia	ylle.jaakma@emu.ee
Maili Jakobson	Estonia	mailijak@ut.ee
Victoria James	United Kingdom	victoria.james@nottingham.ac.uk
Martin Järvekülg	Estonia	martin.jarvekylg@ut.ee
Medine Karadag Alpaslan	Turkey	mediniye@gmail.com
Adriana Karcz	Belgium	adriana.karcz@ugent.be
Sadia Khalid	Estonia	saadia.k27@gmail.com
Elena Kistanova	Bulgaria	kistanova@gmail.com
Magdalena Kowalik	Poland	m.kowalik@pan.olsztyn.pl
Karolina Krajza	Poland	k.krajza@pan.olsztyn.pl
Zuzanna Kunicka	Poland	zuzanna.kunicka@uwm.edu.pl
Beata Kurowicka	Poland	beata.kurowicka@uwm.edu.pl
Arina Lavrits	Estonia	i10l04@ut.ee
Nick Leslie	United Kingdom	n.r.leslie@hw.ac.uk
Freddy Lättekivi	Estonia	freddy.lattekivi@ut.ee
Antonio Marcilla	Spain	antonio.marcilla@uv.es
Tihana Maric	Croatia	tihana.maric@mef.hr
Getnet Midekessa	Estonia	getnet_balcha@yahoo.com
Aleksandra Miłobędzka	Poland	aleksandra.milobedzka@fundacjabirn.pl
Joana Miranda	Portugal	jmiranda@ff.ul.pt
Milena Mourdjeva	Bulgaria	milena_mourdjeva@abv.bg
Marta Muñoz Llamosas	Spain	mmunoz@serida.org
Anne Navarrete Santos	Germany	a.navarrete-santos@medizin.uni-halle.de

Monika Nõmm	Estonia	monika.mm@emu.ee
Mandy Peffers	United Kingdom	peffs@liv.ac.uk
Vanessa Petroni Magri	Malta	vanessa.petroni@um.edu.mt
Stefan Przyborski	United Kingdom	stefan.przyborski@durham.ac.uk
Michael Raghunath	Switzerland	ragh@zhaw.ch
Marie Reemann	Estonia	reemannmarie@gmail.com
Ene Reimann	Estonia	ene.reimann@ut.ee
Qurat Ul Ain Reshi	Estonia	quratbiochem786@gmail.com
Markus Rimann	Switzerland	markus.rimann@zhaw.ch
Bernard Roelen	Netherlands	b.a.j.roelen@uu.nl
Annika Scheynius	Sweden	annika.scheynius@ki.se
Maria Schröder	rway	maria.schroder@odont.uio.
James Smith	United Kingdom	james.smith@nottingham.ac.uk
Martina Štampar	Slovenia	martina.stampar@nib.si
Bojana Zegura	Slovenia	bojana.zegura@nib.si
Oliivika Zeiger	Estonia	oliivika@ut.ee
Marcy Zenobi-Wong	Switzerland	zmarcy@ethz.ch
Algirdas Ziogas	Lithuania	algirdas.ziogas@imcentras.lt
Aleksandra Złotkowska	Poland	a.zlotkowska@pan.olsztyn.pl
Aili Tagoma	Estonia	ailisar@gmail.com
Bilge Guvenc Tuna	Turkey	bilgeguv@gmail.com
Liesbeth Tytgat	Belgium	ltytgat@b-phot.org
Janeli Viil	Estonia	aura@ut.ee
Liis Vitsut	Estonia	liis.vitsut@gmail.com
Krzysztof Wrzesinski	Denmark	kwr@celvivo.com
Melek Yüce	Turkey	melek.yuce@omu.edu.tr