Session 14: Biomaterials, cells and their interactions

Lectures

L.14.1

Bacterial engineers – using microbes to engineer valuable biomaterials

Iza Radecka[1], Fideline Tchuenbou-Magaia[1], Mattia Parati[1], Abhishek Gupta[2], Ibrahim Khalil[1], Barbara Mendrek[3], Grazyna Adamus[3], Marek Kowalczuk[3]

[1]Faculty of Science & Engineering, University of Wolverhampton, United Kingdom; [2]Faculty of Education, Health and Wellbeing, University of Wolverhampton, United Kingdom; [3]Centre of Polymer and Carbon Materials, Polish Academy of Sciences, Poland

Iza Radecka <i.radecka@wlv.ac.uk>

Mountains of waste, including plastic packaging, medical plastic wastes are buried in landfill sites around the world each year. This continues to pose a growing challenge for authorities at both the local and national level, consequently there is a demand for biodegradable materials.

In that context, bacterial polymers have an enormous potential as they can be produced from renewable biogenic resources under well controlled conditions, and they are efficiently biodegraded. Moreover, their expected impact is not simply limited to easing the environmental problems inevitably associated with their production, but also in reducing dependency on fossil-fuel based polymers. Over the past decades many useful biopolymers originating from various types of microorganisms have been reported. Ongoing research has increased rapidly the number of possible applications of these biopolymers, ranging from food additives, biomedical and pharmaceutical systems, agricultural mulching materials to biodegradable packaging and even some electronic components.

However, there are still challenges in developing biodegradable, high performance bacterial biomaterials. Attempts are therefore being made to find new ways in which to increase the efficiency of microbial synthesis of biomaterials. This presentation will describe the significant contribution that the Biopolymer Research Group at the University of Wolverhampton, together with collaborating institutions, is making towards these global issues.

Oral presentations

O.14.1

Development of a Porcine Decellularized Extracellular Matrix (dECM) Bioink for 3D Bioprinting of Meniscal implants: formulation, characterization and biological evaluation

Filip Porzucek[1], Monika Mankowska[1], Julia Anna Semba[1,2], Piotr Cywoniuk[1], Adam Augustyniak[1], Anna Maria Mleczko[1], Tomasz Szymański[1,2], Adam Aron Mieloch[1], Jakub Dalibor Rybka[1]

[1]Center for Advanced Technology, Adam Mickiewicz University, Poznan, Poland; [2]Faculty of Biology, Adam Mickiewicz University, Poznan, Poland

Jakub Dalibor Rybka <jrybka@amu.edu.pl>

Decellularized extracellular matrix (dECM) is an excellent bioink component, closely mimicking native tissue environment including structural, mechanical, and biomolecular properties. In this study, we propose a novel method for bioink formulation utilizing dECM obtained from porcine menisci, specifically designed for the 3D bioprinting of meniscal implants. The decellularization protocol includes a detailed description of the whole process, including supercritical CO_2 extraction ($scCO_2$). The obtained dECM was subjected to DNA, GAG, and collagen content analyses. A microbiological study was performed to confirm the dECM sterility after $scCO_2$ extraction. Subsequently, the rheological properties of various bioink compositions containing dECM, alginate, gelatine, or cellulose nanocrystals were evaluated. Selected bioinks were used for printing accuracy measurement and SEM imaging. For the biological study, human adipose-derived mesenchymal stem cells (hMSC-AT) cultured in monolayer or spheroids were mixed with bioink and 3D bioprinted into constructs. The cell viability within the constructs was analyzed using LIVE/DEAD assay at 1, 10, 20, and 30 days post bioprinting. At the same time intervals, RT-qPCR analysis of selected genes (*COL1A1, COL6A1, COL10A1, COMP, CDH2, SMAD2, SOX9, RUNX2, SOX5, HIF1A*) was performed. In summary, this study provides an in-depth description of dECM production and formulation into bioink for meniscus tissue engineering, with a detailed rheological and biological evaluation.

O.14.2

Death pathways of *Candida albicans* cells after the action of Venetin-1 nanoparticles from the coelomic fluid of *Dendrobaena veneta* earthworm

Sylwia Wójcik- Mieszawska[1], Kinga Lewtak[2], Czaplewska Paulina[3], Fiołka Marta[1]

[1]Department of Immunobiology, Institute of Biological Sciences , Maria Curie-Skłodowska University, Lublin, Poland;; [2]Department of Cell Biology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Lublin, Poland ;; [3]Intercollegiate Faculty of Biotechnology UG-MUG Laboratory of Mass Spectrometry, Gdansk, Poland

Marta Fiołka <marta.fiolka@mail.umcs.pl>

The use of earthworm-derived pharmaceuticals as green medicine is highly developed in China and other Asian countries. Extracts prepared from these invertebrates are used to treat many diseases. Among invertebrates, earthworms are a known source of antibacterial, antifungal and anticancer compounds. The coelomic fluid of the body cavity of earthworms exhibits many types of biological activity, such as antibacterial, proteolytic, hemolytic, hemagglutinating, antifungal and anticancer. In recent years, an increase in resistance to antibiotics in the treatment of fungal infections has been observed. This global problem has created the need to search for new antifungal agents. *Candida albicans* fungus belonging to the order of yeasts is an opportunistic human pathogen. The isolated Venetin-1 nanoparticle has an inhibitory antifungal effect by activating the pathways of apoptosis, necrosis and autophagy of *C. albicans* yeast cells. Fungal cell necrosis and apoptosis were observed by fluorescence microscopy, while autophagy was identified by transmission electron microscopy. The antifungal activity has been confirmed by microscopic, proteomic and flow cytometry methods. The main protein components of the nanoparticle Venetin-1 are lysenin type proteins. Taking into account the effective action against Candida strains in the absence of endotoxicity and cytotoxicity against human normal dermal fibroblasts, the obtained Venetin-1 nanoparticle is a potential antifungal drug.

O.14.3

Design of hydrogel scaffolds for studying stem cells mechanotransduction

Marzena Zychowicz[1], Ewa Walejewska[2], Marcin Heljak[2], Wojciech Święszkowski[2], Leonora Bużańska[1]

[1] Department of Stem Cell Bioengineering , Mossakowski Medical Research Institute, Polish Academy of Sciences; [2] Biomaterials Group, Materials Design Division, Faculty of Materials Science and Engineering, Warsaw University of Technology

Marzena Zychowicz <mzychowicz@imdik.pan.pl>

The survival and function of cells depend greatly on the physical properties of their microenvironment and the mechanical stimuli they experience. Mechanotransduction is a cellular process that involves receiving mechanical signals and converting them into biochemical information. To effectively grow cells, hydrogels should closely mimic the natural microenvironment. In this study, the properties of hydrogel scaffolds designed for culturing: i) neural stem cells derived from induced pluripotent stem cells, and ii) mesenchymal stem cells obtained from Wharton's Jelly were evaluated. Two types of hydrogel scaffolds were then prepared: i) fibrin- and ii) natural-extracellular-matrix-based, and analyzed in terms of swelling, compressive modulus, viscoelastic properties, and internal morphology. The distribution of the YAP/TAZ-mechanotransducing complex within the cells was assessed. The stiffness and viscoelastic properties of biomaterials varied depending on the type of hydrogel and the presence of cells. Additionally, the culture dimensionality influenced the localization of the YAP/TAZ complex within the cells. These findings, along with our previous results, suggest that cells are sensitive to changes in environmental dimensionality, and the hydrogel scaffolds used in this study possess appropriate mechanical characteristics for stem cell applications.

Acknowledgements

This work was supported by National Science Centre grant Miniatura-3 (2019/03/X/NZ3/01109) and statutory funds to MMRI PAS.

Posters

P.14.1

Seeds of Virginia mallow as a source of antifungal and antibacterial compounds

Kinga Lewtak[1], Paulina Czaplewska[2], Weronika Sofińska-Chmiel[3], Marta Fiołka[4]

[1]Department of Cell Biology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Lublin, Poland; [2]Intercollegiate Faculty of Biotechnology UG-MUG Laboratory of Mass Spectrometry, Gdańsk, Poland; [3]Analytical Laboratory, Institute of Chemical Sciences Maria Curie-Skłodowska University, Lublin, Poland; [4]Department of Immunobiology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Lublin, Poland

Kinga Lewtak <kinga.lewtak@mail.umcs.pl>

The current state of civilization diseases, as mycoses and mycobacteriosis, is the result of the growing resistance of microorganisms to available antibiotics, but also their significant toxicity. Therefore, medicine based on substances of natural - plant origin can become a solution. The analysis of the antimicrobial activity of Virginia mallow (*Sida hermaphrodita*) seed extract included the study of the effect of seed extract, and extract fractions on *Candida albicans* and *Mycobacterium smegmatis* cells. The analyzes performed using light, fluorescence, transmission, and atomic force microscopy as well as spectroscopy methods showed that the extract had significant effect on mycobacteria cells, by reducing the viability of the bacteria and inducing changes in the cell wall structure. It was also found that seed extract and its fractions reduced the metabolic activity of *C. albicans* cells, and affected the yeast cell wall causing numerous deformations on the yeast cell wall surface, an increase in the average thickness and changes in chemical composition of cell wall. Biochemical and proteomic characterization revealed structural, storage and enzymatic proteins and peptides typical of seeds in the extract. In the protein profile of the extract, antimicrobial proteins and peptides, identified as vicillins, and lipid transport proteins were also determined. The conducted analyzes indicate that *S. hermaphrodita* extract may be a potential source of antimicrobial pharmaceuticals.

P.14.2

BMP-2 with ERK inhibitor and Phenamil effectively enhance osteogenesis of human adipose-derived stem cells cultured on SrO or ZnO modified bioactive glass-PLGA composites in static and dynamic cultures

Karolina Truchan[1], Barbara Zagrajczuk[2], Katarzyna Cholewa-Kowalska[2], Anna Maria Osyczka[1]

[1]Jagiellonian University, Faculty of Biology, Institute of Zoology and Biomedical Research, Department of Biology and Cell Imaging, Krakow, Poland; [2]AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Department of Glass Technology and Amorphous Coatings, Krakow, Poland

Karolina Truchan <karolina.truchan@doctoral.uj.edu.pl>

Strategies to improve bone regeneration therapies with adult stem cells are still in high demand. Adipose-derived mesenchymal stem cells (ASC) offer an alternative source of multipotent cells to bone marrow stem cells (BMSC). However, the osteogenic potential of ASC is reportedly lower than BMSC. Here, we present new strategies for osteogenic differentiation of human ASC by combining culture on bioactive composites, treatment with chemical compounds and application of dynamic culture conditions. We cultured human ASC cell line (ASC52telo, ATCC) on PLGA-based composite sheets consisting of 50% wt. sol-gel bioactive glasses in the SiO_2-CaO-P_2O_5 system modified with either ZnO or SrO. Cells were treated with bone morphogenetic protein 2 (BMP-2), ERK kinase inhibitor, Phenamil, and subjected to fluid shear stress on a standard horizontal rocker. Notably, after 3-day culture under such conditions, ASC52telo cells increased mRNA levels for osteocalcin, osteoprotegerin and osteonectin, all markers related to osteogenesis. After 7-day culture as above and subsequent transfer of the cells to tissue culture plastic, ASC52telo cells produced significant amounts of mineralized extracellular matrix within the following 14 days. Our new osteogenic differentiation strategies for ASC offer several new possibilities of *in vivo* ASC delivery for bone regeneration therapies. Both composites and/or chemically/mechanically treated ASC (together or separately) are suitable for *in vivo* application.

P.14.3

Growth of mesenchymal stem cells (MSC), gingival fibroblasts (GF) and periodontal cells (PDL) on semi-crystalline PEEK and PEKK polymers as potential materials in dental implantology – preliminary study

Agata Poniewierska-Baran[1,2], Małgorzata Tomasik[3], Katarzyna Grocholewicz[3], Ewa Sobolewska[4], Andrzej Pawlik[2], Marcin Królikowski[5]

[1]Institute of Biology, University of Szczecin, Szczecin, Poland; [2]Department of Physiology, Pomeranian Medical University, Szczecin, Poland; [3]Department of Integrated Dentistry, Pomeranian Medical University, Szczecin, Poland; [4]Department of Prosthodontics, Pomeranian Medical University, Szczecin, Poland; [5]Department of Manufacturing Technology, ZUT, Szczecin, Poland
Agata Poniewierska-Baran <agata.poniewierska-baran@usz.edu.pl>

The 3D printing has revolutionized the possibilities of producing individual implants for bone reconstruction and recently also elements of dental implants made of durable polymer materials characterized by very good biocompatibility in the tissue environment, thus constituting an alternative to titanium implants. **The aim** of the project was to study the proliferation of cells isolated from oral tissues on selected polymers PEEK (poly-ether-ether-ketone) and PEKK (poly-ether-ketone-ketone) printed in 3Dtechnology, with mechanical properties similar to bone. **Methods:**3 cell lines from oral tissues were used 1)mesenchymal stem cells-MSC 2)gingival fibroblast-GFs and 3)periodontal ligament-PDLs. Cells were seeded in 24well culture plates with PEEK&PEKK rings for 0-72h. Cells were counted by FACS at each time point. **Results:**Interestingly, cultures on rings made of PEEK&PEKK showed a difference in the proliferation of these cell lines, which may indicate the preference of oral tissues to grow & cover implants, depending on the type of material used for their production. Human GF & MSC grew significantly faster on PEEK compared to PEKK on which proliferation was stopped. On the PEKK, only PDL cells proliferated, and number of GF&MSC cells on PEKK did not change for 72h. experiment. **Conclusions:**The results of the experiment showed that PEEK is the more preferred material for the proliferation of cells found in the oral cavity and important for dental implantology.

P.14.4

Hybrid support for scalable, high-performance oligonucleotide synthesis

Krzysztof Waligórski[2], Kamil Kamiński[2], Mikołaj Przybyła[2], Katarzyna Kolet[2], Oskar Kołacki[2], Stanisław Trzciński[1], Mateusz Klarek[1], Jolanta Brzezińska[1], Marcin K. Chmielewski[1]

[1]Institute of Bioorganic Chemistry PAS, Poznań, Poland; [2]FUTUREsynthesis sp. z o.o., Poznań, Poland
Marcin Chmielewski <chmielewskimk@ibch.poznan.pl>

The main problem with scalability of oligonucleotides (ON) synthesis is the use of different apparatuses, methods, reagents, and supports in small (up to 10μmol) and large-scale synthesis. Supports based on polystyrene cores, which are used in large-scale synthesis, are not suitable for small-scale driven by gas pressure due to swelling and increasing resistance. Conversely, Controlled Pore Glass (CPG) based supports have insufficient loadings to be used in large-scale synthesis.

To bridge the loading gap and remove the problem of swelling, we development of supports based on a solid core bonded with a polymer layer that will provide a developed surface area and a binding site for growing ON. A solid base should prevent clogging in small-scale synthesis by limiting swelling in organic solvents, and polymer coating should provide surface area than the "naked" CPG, and consequently more space for loadings.

Synthetic polymers were tested to develop a hybrid support and small-scale solid-phase synthesis was performed. The amount of crude ONs were often higher than the commercially CPG, and RP-HPLC analysis of the synthesized ON showed high quality of the obtained material.

Further research is needed to be done to better understand the structure of polymers and how it changes during the different steps of solid phase ON synthesis. Furthermore, improvements need to be done in terms of the final loading of the hybrid support in order to obtain a truly commercially attractive product.

P.14.5

The new innovative method for the early detection of a blood-borne biomarker of an invasive fungal infection

Magdalena Mnichowska-Polanowska[1], Bartosz Wojciuk[2], Ewa Mijowska[3], Krzysztof Cendrowski[3], Barbara Dołęgowska[4]

[1]Independent Laboratory of Medical Microbiology, Pomeranian Medical University in Szczecin, Poland; [2]Department of Immunological Diagnostics, Pomeranian Medical University in Szczecin, Poland; [3]Department of Nanomaterials Physicochemistry, West Pomeranian University of Technology, Szczecin, Poland; [4]Department of Laboratory Medicine, Pomeranian Medical University in Szczecin, Poland

Magdalena Mnichowska-Polanowska <magdalena.mnichowska.polanowska@pum.edu.pl>

Due to common overdiagnosis, the clinical identification of invasive fungal infection (IFI) requires improvement. This currently results in the increased antifungal treatment, prolonged hospital stay and high mortality. The new approach to IFI diagnosis considers 'bedside assay' using biosensor technology in order to provide more effective diagnosis and more sustainable therapeutic decisions. The aim of this research was to develop the electrode modified (functionalized) with single-walled carbon nanotubes (SWCNTs) and recombinant human dectin-1 molecule (IFI biomarker receptor) to enable electrochemical detection of beta-glucan which is recognized as a IFI biomarker. SWCNTs were functionalized with dectin-1 which formed stable amid bonds, confirmed with infrared and Raman spectroscopy. Further dectin-1-functionalized SWCNT were deposited on the electrode surface, and detected by both Raman spectroscopy and scanning electron microscopy. Preliminary studies on blood-borne β-glucan detection, were carried out in a blood sample with the modified electrodes using impedance spectroscopy. The nanotubes-and dectin-1-modified electrode designed in this study enabled the detection of the IFI biomarker in the blood samples. Measurement of the IFI biomarker using this electrode appears sensitive, selective and rapid detection system, useful for the rational bedside management of IFI, especially with regard to the proper decision either to initialize or abandon antifungal treatment.