MARTIN-LUTHER-UNIVERSITÄT
HALLE-WITTENBERG

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ESAO
European Society for Artificial Organs

ESAO Winter School 2020 Program

Materials, Surfaces & Cells:
Science & Technology for Bio Artificial Organs and Tissue Engineering

Leucorea
Lutherstadt Wittenberg, Germany
February 26-29, 2020

Dear Participants of the ESAO Winter School 2020,

It is our great pleasure to welcome you to this year´s Winter School of the European Society for Artificial Organs. Our Winter Schools have a long tradition and follow the idea of contributing to the education and training of Master- and PhD students as well as Post-Docs, but also of teaching the teachers.

Every year, a different topic is in the focus of the school. Since many ESAO members and participants of our annual congresses have s strong interest in the area of material sciences, tissue engineering, and regenerative medicine, we will focus on biopolymers and their application in the above-mentioned areas this year. We will go beyond in vitro studies - which will, however, represent an important part of the meeting, but in a comprehensive manner from bench to bedside with exciting new areas, such as bioprinting and organ-on-a-chip devices.

We were able to attract excellent speakers with impressive track records who made major contributions in their areas of expertise. We also invited members of the next generation of scientists who have already left their footprints in specific disciplines.

The school shall provide an open platform for discussion. We organized a get-together after the opening session and two guided poster sessions, where participants can present their research results and discuss with peers and experienced scientists. We chose a relaxed atmosphere with beverages to support the exchange of ideas and the planning of future collaborations.

You will notice that the region of Wittenberg is not particularly famous for winter sports, but it has a rich and interesting history. The venue, Leucorea, represents remnants of one of the oldest universities in Germany, a place where Martin Luther worked as teacher, and the town Wittenberg is famous as the place from where Protestant Reformation originated.

We organized a guided tour to introduce you to the numerous interesting places in Wittenberg. A free afternoon will give you a chance to visit sights such as the workshop of the painter Lucas Cranach, the Palace and Luther Museum, churches, as well as other interesting places.

Last, but not least, we would like to acknowledge the financial support by the Martin Luther University Halle-Wittenberg and by the Fraunhofer Institute IMWS that allowed us to keep the fees for the Winter School low. We are indebted to the members of the Department of Biomedical Materials, Martin Luther University, for their support in preparing the meeting. We are also thankful for the support by the ESAO Office in Krems, specifically Anita Aichinger, who handled registrations, abstracts, and fees.

We do hope that you will enjoy the meeting with an interesting program in a great atmosphere in a historical environment.

We wish you an unforgettable stay at the ESAO Winter School 2020 in Lutherstadt Wittenberg!

On behalf of the Scientific Committee

Thomas Groth (chair)
Martin Luther University Halle

Nuno Neves
University of Minho

Viktoria Weber
Danube University Krems

Organizers

European Society for Artificial Organs

European Society for Artificial Organs

The ESAO founded 1974 in Geneva is a prominent not-for-profit European organization. The running activities are emerging into an interdisciplinary field of research, development and clinics in which fields of artificial and bioartificial organs, applied biomaterials sciences, tissue engineering and regenerative medicine aim to support, replace or restore the function of tissues and organs. The mission of ESAO is grounded on four key global sustainable, measurable, and science evidence-base pillars: to promote and publish advances in the field (i); to coordinate and develop research (ii); to promote and exchange information (iii) and to promote and coordinate activities with similar European Societies (iv). The ESAO organization issues one official scientific journal, IJAO - The International Journal of Artificial Organs and online News & Updates letters. ESAO clusters its activities in the Annual Congress, held in different European cities, the organization of schools and different working groups. For more details see www.esao.org

Fraunhofer Institute for Microstructure of Materials & Systems Halle

With the goal of increasing both material and economic efficiency, as well as conserving resources, Fraunhofer IMWS is a renowned partner for industry and public authorities. Fraunhofer IMWS addresses any associated questions at the smallest of dimensions, at the microstructural level of materials and systems, and thereby contributes to solutions to global challenges. Within Fraunhofer IMWS, the Department of Biological and Macromolecular Materials focus on the needs of personal care and medical device industry. We perform materials research for personal care products, the development and characterization of biomaterials for medical devices and the functionalization of surfaces. Furthermore, we offer a broad range of services including mechanical, morphological, biological and chemical test methods for directed development and evaluation of future materials. For more details see www.imws.fraunhofer.de/de.html

Martin Luther University Halle-Wittenberg

Education and research with a 500-year-old tradition: Martin Luther University Halle-Wittenberg (MLU) offers a wide range of academic subjects in the areas of humanities, social sciences, natural

sciences and medicine. The oldest and largest university in Saxony-Anhalt was created in 1817 when the University of Wittenberg (founded in 1502) merged with Friedrichs University Halle (founded in 1694). Today the university has around 20,000 students and 340 professors. MLU's academic profile in the field of the humanities is shaped by the core research areas "Enlightenment – Religion – Knowledge" and "Society and Culture in Motion. Diffusion - Experiment - Institution". The university's core scientific research is in "Materials Science – Nanostructured Materials" and "Biosciences – Structures and Mechanisms of Biological Information Processing". Agricultural sciences also play a leading role and Halle's university bears sole responsibility in Saxony-Anhalt for developing this academic profile. The Faculty of Medicine focuses on epidemiology, health and nursing research and research on signal transmission. Here nursing scientists conduct research on an equal footing with physicians. The Dorothea Erxleben Learning Centre is one of the largest teaching clinics for prospective medical professionals. In addition to its main academic focuses, the university offers a range of minor subjects, some of which are only offered in Germany at MLU. The Winterschool 2020 is organized with support from Department Biomedical Materials (BMM), Institute of Pharmacy, Martin Luther University Halle-Wittenberg. More details about BMM can be found here https://bmm.pharmazie.uni-halle.de

Scientific Committee

Thomas Groth	Martin Luther University, Halle, Germany
Nuno Neves	University of Minho, Braga, Portugal
Viktoria Weber	Danube University Krems, Austria

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Venue Leucorea

In 1502, “Frederick The Wise”, Elector of Saxony, founded the Electoral Saxonian State University in Wittenberg with the name LEUCOREA (“leukos oros” = “white mountain” in Greek, a reference to the name of the city of Wittenberg), which would become world-renowned. After the university was granted royal privileges by Maximilian I on 6. July, 1502, the ceremonious inauguration took place on 18. October, 1502. Martin Pollich of Mellerstadt, the personal physician of Frederick The Wise, was made the founding Rector. Since papal confirmation of the university did not occur until 1507, this constituted the first sovereign founding of a university in Germany. University operations commenced with 416 students, learning in rooms of the Augustinian and Franciscan monasteries. The ‘Fridericianum’ college was the first building erected for university purposes, in 1503/04 by the master builder Konrad Pflüger. Many important scholars from different academic faculties can be named, but particularly Martin Luther, starting in 1512, had the greatest effect on Wittenberg. The LEUCOREA became a place of world history when Luther, with his 95 theses on indulgences in 1517, ushered in the Reformation and thus prompted repercussions lasting to the present. With the appointment of 21-year-old Philipp Melanchthon as a professor for the Greek language, who quickly became a close friend and theological confidant of Luther, the young university gained even more prominence. Beginning with his opening lecture in 1518, Melanchthon initiated an extensive reform of the university, which conveyed the close connection between humanism and the Wittenberg Reformation. This, most notably, led to Wittenberg becoming the most well-attended university of the kingdom between 1530 and 1620. The Leucorea merged in 1817 with the Frederic University in Halle. Today, Leucorea is part of Martin Luther University Halle-Wittenberg and used as venue of colloquia, congresses and other meetings. It has several lecture halls, seminar rooms and a cafeteria and is located in the old part of Wittenberg close to many historical sites.

Lutherstadt Wittenberg

Is a town in Saxony-Anhalt, Germany situated on the River Elbe. The town is famous for its close connection with Martin Luther and the Protestant Reformation. Several of Wittenberg's buildings associated with the events, including a preserved part of the Augustinian monastery in which Luther lived, considered to be the world's premier museum dedicated to Luther. Today, Wittenberg is a popular tourist destination, best known for its intact historic centre and various memorial sites dedicated to Martin Luther and Philip Melanchthon added to the UNESCO world heritage list in 1996.
We organized a guided tour through the town to learn about its history and most important buildings.

Scientific Topics

- Biogenic biomaterials
- Engineering of biomaterial surfaces & 3D systems
- Hydrogels, scaffolds, bioprinting, Organ-on-a-Chip
- Stem cell biology and tissue engineering
- Translation from bench to bed site

Lectures

All lectures will be given in the large lecture hall on ground floor. Speakers are asked to provide their presentations on a USB stick to the organizers at the reception desk 15 min before beginning of their sessions. Speakers who intend to use their own computer should also contact the organizers at reception desk 15 min before the begin of session.

Poster Sessions

Two guided poster sessions will be organized in the room 1st floor. Presenters of poster session I (P1 – P15) can mount their posters on February 26 and shall remove them after the poster session I on February 27. Posters for session II (P15-P32) shall be mounted in the morning, February 28 and be removed before the end of the meeting, February 29.
Poster Awards will be given for best three posters.

Social Program

Get-together: After the opening session on February 26, all participant are invited to join the get-together, which be held in the rooms at 1st floor 6.30 p.m. Snacks and beverages will be served.

Some snack and beverages will be provided during **Poster session I & II** at rooms 1st floor on February 27 from 6 p.m. and February 28, 5.30 p.m. on.

We will organize one **guided city tour on February 27, 2 p.m.** to experience the historic city center of Wittenberg with its Schlosskirche (Castle Church) and the famous door where Martin Luther nailed his 95 Theses and other interesting places. Stroll around the streets where Martin Luther and his companions have already been long time ago and experience the spirit of this old town. Please, register for the guided city tour at our reception desk, when you are checking in because we will organize two groups in parallel.

On February 28, after the morning sessions you will have time to visit more interesting places in Wittenberg before we continue with the afternoon session from 4 p.m. on.

Scientific & Social Program

Wednesday, February 26

13.30 – 16.30	**Registration**
16:30 - 16:40	**Welcome & Opening of Winter School** **Chair:** Thomas Groth, Halle (Saale)
16:40 - 17:30	**Opening Lecture 1** **The roles of materials, surfaces and cells on the engineering of different tissues and organs.** Rui Reis, University of Minho, Portugal
17:30 - 18:20	**Opening Lecture 2** **Elastic biomaterials and advanced wound repair** Anthony Weiss, University of Sydney, Australia
18:30 – 20.00	**Get together with buffet & beverages**

Thursday, February 27

Session 1	**Biopolymers I** **Chair:** Christian Schmelzer, Halle (Saale)
08:30 - 09:10	**Synthesis of stimuli-responsive bioactive compounds from polysaccharides** Kai Zhang, University of Göttingen, Germany
09:10 - 09:50	**Polysaccharide chemistry and modification for chemical and photochemical cross-linking reactions** Matthias Schnabelrauch, INNOVENT Jena e.V., Germany
09:50 - 10:20	**Coffee Break**

Session 2	**Biopolymers II** **Chair:** Matthias Schnabelrauch, Jena
10.20 – 11.10	**From natural resources to engineered artificial matrices** Antonella Motta, University of Trento, Italy
11:10 – 11:50	**Elastin analytics and electrospinning - Understanding the biomolecular world with the power of mass spectrometry** Christian Schmelzer, Fraunhofer IMWS Halle, Germany
12:00 – 14:00	**Lunch Break**
14:00 – 16:00	**Guided Tour through Lutherstadt Wittenberg**
Session 3	**Engineering cellular microenvironments I** **Chair:** Antonella Motta, Trento
16:30 – 17:20	**Engineering the cellular microenvironment with biomaterials and growth factors"** Manuel Salmeron Sanchez, University of Glasgow, Scotland
17.20 – 18.10	**Programmable cell instructive materials to control cell morphogenesis in vitro** Paolo Netti, University of Naples, Italy
18:00 -19:30	**Poster Session I with snacks & beverages, Seminar room 1st floor** **Chairs:** Viktoria Weber & Gerado Catapano

Friday, February 28

Session 4	**Cellular microenvironments II** **Chair:** Nuno Neves, Bragha
08:30 – 09:20	**Immunological challenges in regenerative medicine** Hans-Dieter Volk, Charité, Universitätsmedizin Berlin, Germany

09:20 – 10:00	**Extracellular vesicles and their roles in inflammation and regeneration** Viktoria Weber, Danube University Krems, Austria
10:00 – 10:30	**Coffee Break**
Session 5	**Cells, Materials & Scaffolds I** **Chair:** Manuel Salmeron Sanchez, Glasgow
10:30 – 11:20	**Cell-rich microstructures in bottom-up tissue engineering** João Mano, University of Aveiro, Portugal
11.20 – 12.10	**Application of improved gellan gum-based scaffolds for Tissue Engineering in vivo** Gilson Khang, Chonbuk National University, South Korea
12.10 – 12:50	**The interplay between structural and transport properties of the scaffold and cells metabolism in bioengineered tissues** Gerardo Catapano, University of Calabria, Italy
12:40 - 14:30	**Lunch Break**
14:30 – 16:00	**Free time for sightseeing in Wittenberg**
Session 6	**Engineering cellular microenvironments II** **Chair:** Pedro Baptista, Zaragoza
16.00 – 16:50	**Polymeric hydrogels: From fundamental physicochemistry to tissue engineering** Gloria Gallego-Ferrer, Polytechnical University Valencia, Spain
16.50 – 17.30	**Biomaterials, Porous Scaffolds and Cells for Advanced Therapy** Nuno Neves, University of Minho, Portugal

17:30 – 19:00 **Poster Session II with snacks & beverages, Seminar room 1st floor**
Chairs: Nuno Neves & Gloria Gallego Ferrer

Saturday, February 29

Session 7 **Translation to clinical application**
Chair: Gloria Gallego Ferrer, Valencia

08:30 – 09:10 **Pioneering solid organ bioengineering: past, present and future perspectives, and the politics within**
Pedro Baptista, University of Zaragoza, Spain

09:10 - 09:50 **Clinical translation of fibrin agarose-based artificial tissues**
Miguel Alaminos, University of Granada, Spain

09:50 - 10:20 **Coffee Break**

Session 8 **Bioprinting and Organ-on-Chips**
Chair: Viktoria Weber, Krems

10:20 - 11:10 **Bioprinting cell laden building blocks for Tissue Engineering applications**
Claudio Migliaresi, University of Trento, Italy

11:10 - 11:50 **Biology-inspired microphysiologial systems to advance medicines**
Eva Dehne, TissUse GmbH Berlin, Germany

11.50 – 12:30 **Additive manufacturing and bioprinting techniques for tissues and organs.**
Carlos Mota, University of Maastricht, The Netherlands

12:45 - 13:15 **Poster Award Ceremony and Closing remarks**
Thomas Groth, Halle, Germany

Poster presentations – Seminar room 1st floor

Poster Session I, February 27, 6 p.m. – 7.30 p.m.

P1 Discovery and Characterization of Bovine Elastin Splice Variants on the Protein Level
Mina H.N. Rizk, T. Hedtke, C.E.H. Schmelzer; Halle (Saale), Germany

P2 Synthesis of thermo-responsive biological cellulose-based GAGs mimetic materials
Kui Zeng, Falko Doberenz, Thomas Groth, Kai Zhang[a]; Göttingen, Germany

P3 Development of 3D printing of modified chondroitin sulfate/hyaluronic acid hydrogels for engineering of soft tissues
Sophie Bendix, Reema Anouz, Christian Willems, Thomas Groth; Halle (Saale), Germany

P4 Biomembranes for tissue engineering: a Raman spectroscopy study of freeze-dried hAM
Sara Leal Marin, Olena Pogozhykh, Constanca Figueiredo, Birgit Glasmacher, Oleksandr Gryshkov; Hannover, Germany

P5 Application of hyaluronic acid microsphere for promotion of differentiation of stem cells
Yujin Park, Kyoung Hwan Park, Kang Moo Huh, Sun-Woong Kang; Dajeon, Korea

P6 The effects of hexanoyl glycol chitosan on an ultra-low cell adhesion material for three-dimensional multicellular spheroids culture
Truong Thuy Trang, Kyoung Hwan Park, Kang Moo Huh, Sun-Woong Kang; Dajeon, Korea

P7 The experimental model of 3D tumor cell growth using poly(3-hydroxybutyrate) microspheres
K.A. Menshikh, V.V. Voinova, T.K. Makhina, G.A. Bonartseva, K.V. Shaitan, A.P. Bonartsev; Moscow, Russia

P8 Hydrogel-poly(vinylidene fluoride) microspheres platform for mesenchymal stem cell differentiation
Maria Guillot-Ferriols, María Inmaculada García-Briega, Senentxu Lanceros-Méndez, José Luis Gómez-Ribelles, Gloria Gallego-Ferrer; Valencia, Spain

P9 Calcium phosphates for bone tissue engineering from biogenic resources
Francesca Cestari, Giovanni Chemello, Anna Galotta, Vincenzo M. Sglavo, Antonella Motta; Trento, Italy

P10 Hydrothermal microwave synthesis of nano-Hydroxyapatite with strictly controlled particle sizes - for biomedical applications
U. Szałaj[1], A. Chodara, S. Dąbrowska, J. Wojnarowicz, T. Chudoba, S. Kuśnieruk, Witold Łojkowski; Warsaw, Poland

P11 Novel surface coatings as biocompatible reservoirs to deploy BMP-2 for bone regeneration
Reema Anouz, Alexandros Repanas, Elisabeth Schwarz, Thomas Groth; Halle (Saale), Germany

P12 Effect of Metal ions on Multilayer Properties and Cell Response
Hasna Kindi, Thomas Groth; Halle (Saale), Germany

P13 Embedding novel cationic liposomes on the terminal layers of polyelectrolyte multilayer system to study the controlled release on myoblast cells
Yazmin Angelina Brito Barrera, Christian Wölk, Thomas Groth; Halle (Saale), Germany

P14 Fabrication of antibacterial membranes with increased osteoconductivity for periodontal tissue regeneration
Julia Higuchi, Bartosz Woźniak, Jacek Wojnarowicz, Urszula Szałaj, Witold Łojkowski

P15 Antimicrobial multilayer coatings based on Collagen and Tannic acid
Muhammad Haseeb Iqbal, André Schroder,[b]Halima Kerdjoudj, Christian Njel, Bernard Senger, Vincent Ball, Florent Meyer, Fouzia Boulmedais,[1]; Strasbourg, France

Poster Session II, February 28, 5.30 p.m. – 7 p.m.

P16 Bioabsorbable electrospun materials for *in situ* drug release in wound healing applications
Tobias Hedtke, Thomas Groth and Christian E.H. Schmelzer; Halle(Saale), Germany

P17 A universal system to control the unspecific protein and cell adhesion on electrospun polycaprolactone wound dressings
Manuela Garay, Christoph Suscheck, Ulrich Schwaneberg, Cesar Rodriguez-Emmenegger; Aachen, Germany

P18 Free-Standing Multilayer Films as Growth Factor Reservoirs for Wound Dressing Applications
Adrian Hautmann, Devaki Kedilaya, Sanja Stojanović**,** Gurunath Apte, Stevo Najman**,** Thomas Groth, Halle (Saale), Germany

P19 The artificial biofilm based on composite construction from bacterial poly(3-hydroxybutyrate) and alginate seeded with probiotic bacteria
V.V. Voinova, A.A. Dudun, E.A. Akoulina, I.I. Zharkova, D.A. Chesnokova, T.K. Makhina, G.A. Bonartseva, K.V. Shaitan, A.P. Bonartsev; Moscow, Russia

P20 Thermoresponsive polyelectrolyte multilayers of chitosan and PNIPAm-modified cellulose sulfate for the generation of cell sheets for tissue engineering
Falko Doberenz, Kui Zeng, Kai Zhang, Thomas Groth; Halle (Saale), Germany

P21 Development of biogenic thermoresponsive polyelectrolyte multilayers for the application on tissue engineering
Yi-Tung Lu, Kui Zeng, Kai Zhang, Thomas Groth; Halle (Saale), Germany

P22 Surface-Mediated Gene Delivery from functionalized Polyelectrolyte Multilayer Scaffolds in Tissue Engineering
Catharina Husteden, Falko Doberenz, Shashank Reddy Pinapirreddy, Andreas Langner, Thomas Groth, Christian Wölk; Halle (Saale), Germany

P23 Polysaccharide-Based Hybrid Microgel/Hydrogel Systems for Tissue Regeneration
C. Willems, Oleksandra Skorobohatko, M. Muhammad, T. Groth; Halle (Saale), Germany

P24 Human hepatocytes encapsulated in injectable hydrogels of hyaluronic acid and gelatin
Julio Rodríguez-Fernández, Emma Garcia-Legler, Sandra Clara-Trujillo, Mª Teresa Donato, Gloria Gallego-Ferrer, Laia Tolosa; Valencia, Spain

P25 Generation of mature hiPSC derived hepatocytes: a nature-inspired protocol
Joana I. Almeida, Pedro Vicente, Paula M Alves, Pedro M. Baptista, Margarida Serra; Oeiras, Portugal

P26 Efficiency of mononuclear stem cells and statinotherapy in rats with non-alcoholic steatohepatits
Appelhans Olena, Maznichenko Yegor; Odessa, Ukraine

P27 Development of a bio-artificial kidney as a cell-based renal model
Alexandros Englezakis, Elnaz Gozalpour, Karen Coopman, Elisa Mele, Katherine Fenner; Loughborough, United Kingdom

P28 Adaptive *de novo* fibrinolytic hemocompatible membranes for artificial lung assists
Lena Witzdam, Fabian Obstals, Manuela Garay Sarmiento, Oliver Grottke, Cesar Rodriguez-Emmenegger; Aachen, Germany

P29 Covalent immobilization and multilayer formation of glycosaminoglycans and their anti-inflammatory mechanism of action
Hala AlKhoury, Adrian Hautmann, Frank Erdmann, Guoying Zhou, Sanja Stojanović, Stevo Najman, Thomas Groth; Halle (Saale), Germany

P30 Immunological Potential of Mesenchymal Stem Cells
Markus Pasztorek, Eva Rossmanith, Christoph Mayr, Fabian Hauser, Jaroslaw Jacak, Andreas Ebner, Viktoria Weberand Michael B. Fischer; Krems, Austria

P31 Functional capacities of extracellular vesicle micro-RNA in autologous blood-derived products
Alexander Otahal, Olga Kuten-Pella, Karina Kramer, Christoph Stotter, Zsombor Lacza, Stefan Nehrer, Andrea De Luna; Krems, Austria

P32 Use of Size-Exclusion Chromatography for the Removal of Co-Enriched Factors from Platelet-Derived EVs
Sobha Karuthedom George, René Weiss, Victor U. Weiss, Stephanie Steinberger, Günter Allmaier, Viktoria Weber, Carla Tripisciano; Krems, Austria

Poster abstracts

P1 Discovery and Characterization of Bovine Elastin Splice Variants on the Protein Level

Mina H.N. Rizk[1,2], T. Hedtke[1,2], C.E.H. Schmelzer[1,2]

[1] Fraunhofer Institute for Microstructure of Materials and Systems IMWS, Halle, Germany
[2] Martin Luther University Halle-Wittenberg, Halle, Germany

Elastin is an essential, highly cross-linked extracellular matrix protein responsible for the elasticity of dynamic vertebrate tissues such as aorta, ligaments, skin, lungs, or elastic cartilage. Despite the presence of only one gene in mammals coding for tropoelastin (TE), elastin's monomeric precursor, the formation of multiple isoforms were reported for different spices, including rat, cow, and human. These isoforms are the result of extensive alternative splicing, which takes place after pre-mRNA transcription of the corresponding TE gene. Although the splicing of elastin has been known for decades, its exact cause and significance for the properties of elastin and the tissues have been elusive. In this study, we aimed to elucidate the diversity of the splicing on the protein level. Thus, we isolated elastin from different bovine tissues, including aorta, ligament, and elastic cartilage, using a gentle isolation method. The pure elastin was then hydrolyzed using three nonspecific elastases; pancreatic elastase, cathepsin G, and matrix metalloproteinase 12. The digests were analyzed using high-resolution tandem mass spectrometry, and the data were searched against a comprehensive database, which was developed for this purpose and comprised known as well as many hypothetical bovine TE isoform sequences with and without probable mutations. A splice variant was considered when certain tandem mass spectra were matching to one or more peptides uniquely assignable to a domain-domain junction of one of the known or hypothetical isoforms in the database.

With this approach we were able to prove on the protein level the presence of different TE domains prone to splicing, which results in the formation of different TE isoforms. We confirmed four splicing events that were earlier reported and present in Swiss-Prot and/or NCBI RefSeq. Moreover, we discovered in this way six further splicing sites that were not previously reported in the literature or any protein databases. It is worth mentioning, that most of these different isoforms were detected in parallel; i.e. they are present within the same tissue and animal. The complexity of the splicing adds to the heterogeneity of the biopolymer elastin.

P2 Synthesis of thermo-responsive biological cellulose-based GAGs mimetic materials

Kui Zeng,[a] Falko Doberenz,[b] Thomas Groth,[b] Kai Zhang[a]

a.Wood Technology and Wood Chemistry, University of Goettingen, Büsgenweg 4, D-37077 Göttingen, Germany
b.Department Biomedical Materials, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Heinrich-Damerow-Strasse 4, 06120 Halle (Saale), Germany

GAGs exhibits important biologic properties. However, GAGs limited by several drawbacks. Cellulose-based heparan sulfate mimetic material with thermos-responsive properties (PNIPAM-CS) was synthesized via multi-steps organic synthesis methods. The PNIPAM-CS was determined via various analysis method, such as, NMR analysis, FT-Raman, Elemental analysis, DLS, and different degree of substitution (DS) of sulfate group and PNIPAM were calculated. What's more, the biological properties of PNIPAM-CS with varies DS of sulfate group were tested via applied into tissue engineering.

P3 Development of 3D printing of modified chondroitin sulfate/hyaluronic acid hydrogels for engineering of soft tissues

Sophie Bendix[a], Reema Anouz[a], Christian Willems[a], Thomas Groth[a,b]

[a] Department Biomedical Materials, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Heinrich-Damerow-Strasse 4, 06120 Halle (Saale), Germany
[b] Interdisciplinary Center of Material Science, Martin Luther University Halle-Wittenberg, 06099 Halle (Saale), Germany

3D printing is a promising strategy for individual tissue repair and regeneration. Bioprinting has the benefits to print an exactly designed structure with living cells. Therefore, it can form an interacting and ingrowing cell system with degradable Matrix. In Future this could replace prostheses, which must be exchanged after a certain time period, and enhance the injury healing.
For 3D bioprinting the bioink properties are important for the printability and cell viability. The aim of this project is to develop a 3D printing procedure and hydrogels, which are applicable for tissue engineering of soft tissue. For the Fabrication of hydrogels thiolated hyaluronic acid (tHA) and thiolated or allylated chondroitin sulfate (tCS/aCS) are used and the different crosslinking strategies can be compared. The crosslinking density, gelling kinetics, rheology and printability from different mixtures are investigated. The printing experiments were implemented with the Inkredible+ CELLINK bioprinter. The degree of functionalization was characterized by 1H-NMR spectroscopy, Ellman's reagent assay and titration. The synthesized glycosaminoglycans showed an effective degree of modification. The crosslinking of the tHA with the aCS can be accelerated with UV-light (365 nm) exposition, whereas crosslinking of two thiolated compounds is only possible in a solvent with a pH-value of 8. It was possible to produce viscous solutions which are usable to print simple shapes with the printer. The next steps will be to determine the rheology details and the biocompatibility. These hydrogels seem to be a promising approach for a new bioink.

P4 Biomembranes for tissue engineering: a Raman spectroscopy study of freeze-dried hAM

Sara Leal Marin[*1], Olena Pogozhykh[2], Constanca Figueiredo[2], Birgit Glasmacher[1], Oleksandr Gryshkov[1]

[1] Institute for Multiphase Processes, Leibniz University Hannover
[2]Institute for Transfusion Medicine, Hannover Medical School.

In tissue engineering, scaffolds are a key element to support cell (in)growth and expansion, especially in 3D. Different materials have been employed to engineer scaffolds mimicking the extracellular matrix of native tissues. One of the most promising is the native membranes, such as the human amniotic membrane (hAM). The hAM has been employed in corneal treatment, wound dressing, gynecology, and other areas due to its antibacterial, antifibrotic, angiogenic properties, and almost no immune response. In order to provide these constructs on demand, they should be efficiently preserved with no alterations in structure and functionality. Most of the techniques currently used for hAM preservation, such as freezing and preservation at ambient temperature could affect its properties. In this work, hAM was freeze-dried under vacuum conditions for 24h (using a Witec 2.0 freeze-drying, Sp scientific, Germany) as a promising preservation approach. The chemical composition of the membrane was assessed using Raman spectroscopy in order to verify the preservation of the main components of the hAM, such as collagen and other extracellular matrix components as proteoglycans in the freeze-dried hAM.

Acknowledgments: This work was supported by the German Academic Exchange Service (DAAD, project number 91725466) in the framework of the Research Grants - Doctoral programmes in Germany.

P5 Application of hyaluronic acid microsphere for promotion of differentiation of stem cells

Yujin Park[1,2], Kyoung Hwan Park[1,2], Kang Moo Huh[1], Sun-Woong Kang[2,*]
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Stem cells are attracting attention in various fields because they can differentiate into various cells. Spheroid cultures are often utilized for chondrogenic and adipogenic differentiation. However, spheroid culture has the limitation that necrotic cores are formed when nutrients and oxygen is not sufficient to reach the into spheroid. To overcome these limitations, hydrogels have been used in spheroid culture. In particular, hyaluronic acid (HA) is receiving more attention because of their excellent biocompatibility, biodegradability, and permeability of oxygen and nutrient. In this study, we used HA hydrogel to promote chondrogenic and adipogenic differentiation of stem cells in spheroid culture. To that end, HA particles were fabricated by the W/O emulsion method. HA particles were embedded between cells for spheroid formation. The spheroid with HA particles showed higher chondrogenic and adipogenic differentiation efficacy than that of conventional spheroid culture methods. This culture method could be used in various fields.

P6 The effects of hexanoyl glycol chitosan on an ultra-low cell adhesion material for three-dimensional multicellular spheroids culture

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Cell culture is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the normal physiology and biochemistry of cells. In the midst of various cell culture methods, 3D cell culture systems have gained increasing interest in complex metabolism and is imperative to develop in vitro cell-based systems that can more realistically mimic the in vivo cell behaviors and provide more predictable results to in vivo tests. However, 3D cell culture plate manufacturing methods are relatively complex, time-consuming, labor-intensive and expensive. Therefore, establish better 3D cell culture method is proposed in in vivo cell-based systems. This study was aimed to determine a manageable, productive and economical 3D cell culture method. Accordingly, we demonstrate that the low cell-adhesion property of hexanoyl glycol chitosan was applicable for forming homogeneous 3D cell spheroid on micropatterned dish and proved no fusion between spheroids comparing to other conventional plates.

P7 The experimental model of 3D tumor cell growth using poly(3-hydroxybutyrate) microspheres

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Three-dimensional cell culture systems are considered as a link between two ways of anticancer drug testing: in vitro screening models and in vivo drug testing on laboratory animals. The aim of this work was to create 3D cell spheroids on microspheres made from poly(3-hydroxybutyrate) (PHB).

To obtain porous microspheres with various diameters, the water phase/oil phase/water phase (W/O/W) method was used, followed by washing out the porogen. An aqueous solution of ammonium carbonate was used as a porogen because of its ability of thermal decomposition to ammonia and carbon dioxide.

To obtain stable spheroids, HEp-2 cells (human laryngeal cancer) were cultured together with porous PHB microspheres in wells coated with 1% agarose gel under constant stirring at a temperature of 37°C and 5% CO2 content. The dynamics of cell division and growth in spheroids was monitored by MTT analysis, the morphology of spheroids was assessed visually by histological methods, as well as by scanning electron (SEM) and confocal microscopy.

The growth of spheroids peaked on the 7th day of cultivation, after which the number of living cells decreased both in the control (cell spheroids) and in the experiment (cell spheroids with microspheres). Moreover, during cultivation with microspheres with a diameter of 50-100 μm, more active proliferation of cells was observed compared to the control and spheroids with microspheres over 100 μm. SEM studies also showed better cell attachment and aggregation with microspheres with smaller diameters.

The described approach to creating 3D cell models from tumor cells using PHB is promising for obtaining systems for testing antitumor drugs. A more complete understanding of the mechanisms will be achieved through analysis of the expression of various transcription factors and during the scaling of the process via cultivation in a larger volume with a constant circulation of the growth media.

The work was supported by Russian Foundation of Basic Research, project #18-29-09099. The equipment of the User Facilities Centers of MSU and RCB RAS were used in the work.

P8 Hydrogel-poly(vinylidene fluoride) microspheres platform for mesenchymal stem cell differentiation

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Bone is a highly heterogeneous tissue, therefore creating a culture platform that mimics its environment is a great challenge. Bone is a piezoelectric tissue and this property is produced by the collagen fibres that form its extracellular matrix. Our study is based on the hypothesis that electrical stimulation, as occurs in native tissue, is a key factor in mesenchymal stem cell differentiation towards the osteogenic lineage. Moreover, a three-dimensional environment will more effectively recreate in vivo conditions than a two-dimensional one. For this reason, our proposal consists of a three-dimensional environment, a gelatin hydrogel in which we have embedded poly(vinylidene fluoride) (PVDF) microspheres, with and without magnetostrictive nanoparticles (cobalt ferrite - CFO) and human mesenchymal stem cells (hMSCs). External magnetic stimulation will produce nanoparticles deformation, deforming the PVDF matrix and producing an electrical signal, which will stimulate hMSC differentiation towards the osteogenic lineage.

PVDF microspheres were obtained using electrospray technique and were physico-chemically characterized. Fourier Transform Infrared Spectroscopy allowed calculating the percentage of different crystalline phases present in the material. Our microspheres had about 85% of beta phase. The degree of crystallinity was characterized by differential scanning calorimetry, being of approximately 60%. The magnetic properties of the microspheres were obtained with a vibrating-sample magnetometer that provided a content of CFO of about 9%. Field emission scanning electron microscopy was carried out to characterize the spheres morphology. Histological slices confirmed the homogeneous distribution of the microspheres in the hydrogel.

hMSC were cultured within the 3D construct, to check the cytotoxicity of the biomaterial. hMSC proliferation was studied at 1, 3 and 7 days, by MTS assay ((4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium). After performing the cultures we can assert that the microspheres are not cytotoxic due to the fact that proliferation is similar to hydrogels without microspheres.

These results are promising for the next phase, which will be the stimulation of the cells through electromagnetic fields.

Acknowledgment to the Spanish Ministry through the MAT2016-76039-C4-R project (including the FEDER financial support).

P9 Calcium phosphates for bone tissue engineering from biogenic resources

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Calcium orthophosphates, in particular hydroxyapatite (HA, Ca10(PO4)6(OH)2), are important for bone tissue engineering because they represent the mineralized part of the natural bone. However, bone differs from pure synthetic materials because it is a non-stoichiometric calcium-deficient phosphate containing additional ions and trace elements. Recent works showed that HA more similar to the bone tissue can be extracted from biological resources.

In this study, cuttlefish bones, mussel shells and chicken eggshells were used as biogenic precursors to synthesize calcium phosphates via wet mechanosynthesis and successive drying in an oven. Different process variables were studied, such as milling time, pH and drying temperature. The as-synthesized compound resulted to be nanocrystalline calcium deficient hydroxyapatite (CDHA), with carbonate ions substitutions and the presence of many trace elements like Na+, Mg2+, Sr2+ and K+.

After consolidation by conventional sintering at about 1000 °C, the materials have been subjected to biological evaluation. The sintering process was found to affect the phase composition, in fact in some cases CDHA was transformed or partially transformed into β-TCP (beta tricalcium phosphate), which is another calcium phosphate of biological interest. Biological tests showed that all materials promote bone cells adhesion and proliferation, therefore being promising materials for bone tissue engineering.

P10 Hydrothermal microwave synthesis of nano-Hydroxyapatite with strictly controlled particle sizes - for biomedical applications

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Hydroxyapatite (HAP, Ca10(PO4)6(OH)2) is an inorganic component of bones and teeth. Hydroxyapatite possesses exceptional biocompatibility and bioactivity properties with respect to bone cells and tissues, probably due to its similarity with the hard tissues of the body. Nowadays hydroxyapatite is one of the most often applied bio-nanomaterials, e.g. in bone implants, scaffold layers, drug delivery agent, dental materials.

This work concerns the unique, green process of microwave synthesis enabling strict control of the size of hydroxyapatite (GoHAP™) nanoparticles in the range of 9±1 to 42±4 nm, thanks to the control of synthesis parameters such as time, pressure and temperature. Full characterization of GoHAP™ nanoparticles and similarity to HAP particles contained in natural bones has been demonstrated. Characterization of nanoparticles was carried out, using methods such as SEM, TEM, XRD, SSA measurement by BET method, skeletal density measurement by helium pycnometry method according to ISO 17025 standards.

Microwave synthesis allows easily and precisely control the grain size of nanoparticles. The size control of HAP nanoparticles gives the possibility of better selection of the material properties for various applications. GoHAP™ particles with a size of 42±4 nm can be successfully used, e.g. in the treatment of dental enamel due to its almost identical crystal structure compared to HAP naturally contained in dental enamel. While GoHAP™ particles of 9±1 nm are almost identical to HAP particles contained in bones, therefore their use in bone tissue regeneration is proposed.

P11 Novel surface coatings as biocompatible reservoirs to deploy BMP-2 for bone regeneration

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Objectives: This study was aimed to fabricate various layer-by-layer (LbL) systems using glycosaminoglycans (GAGs) with capability to bind BMP-2 specifically in order to control osteogenic differentiation of cells by biocompatible release systems.
Methods: Heparin, chondroitin sulfate and their oxidized forms as polyanions were combined with chitosan and collagen I as polycations to form various multilayer coatings on model materials with getting advantage of the intrinsic cross-linking formed between oxidized glycosaminoglycans (GAGs) and polycations to improve multilayer stability and affect the release of BMP-2. The myoblast cell line C2C12, which can differentiate into osteoblasts was seeded on 5 µg/mL BMP-2 loaded multilayers. Cell viability, adhesion, osteogenic differentiation and BMP-2 release were investigated.
Results: C2C12 cells cultured directly on the top of multilayers showed that particularly BMP-2 loaded multilayers made of oxidized GAGs promoted an osteogenic differentiation that was nearly comparable to the positive control, when 5 µg/mL BMP-2 was added directly to the medium. Interestingly, the BMP-2 had synergistic effect on cell adhesion and spreading. BMP-2 in oxidized chondroitin sulfate multilayers was successfully loaded to the layers, sustainably released over time and affected cell differentiation more than the soluble BMP-2.
Discussion: The results show that oxidized GAGs forming intrinsically cross-linked multilayers are useful as reservoirs for sustained release of BMP-2 in which the intrinsic cross-linking affected BMP-2 release, improved multilayers stability due to the resulting stiff surface compared to the native ones, supported cell adhesion, proliferation and subsequent differentiation. This can pave the way for coating implants and scaffolds for repair and regeneration of bone fractures.
Acknowledgements: This work was supported by Bundesland Sachsen-Anhalt in the frame of Leistungszentrum Bio-und Systemtechnik and the International Graduate School AGRIPOLY.

P12 Effect of Metal ions on Multilayer Properties and Cell Response

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Polyelectrolyte multilayers (PEM) prepared by layer-by-layer technique made of polysaccharides hyaluronan (HA) as polyanion and chitosan (CHI) as polycation were additionally cross-linked by transition metal ions (Cu+2, Co+2, Ca+2 and Fe+3). The metal ions were applied to cause coordination-based intrinsic cross-linking of functional groups like amino and hydroxyl groups [1] to modulate physical properties and bioactivity of multilayers to control adhesion and function of mesenchymal murine C3H10T1/2 embryonic fibroblasts. Characterization of multilayer formation and surface properties was done by different analytical methods, which showed changes of wetting, thickness and mechanical properties of multilayers depending on concentration and type of transition metal ion. Most interesting, however was the finding that metal ions like Fe3+ promoted adhesion and spreading of C3H10T1/2 cell greatly on the less adhesive HA/CHI multilayer system while intrinsic cross-linking by Cu2+ reduced adhesion and spreading of cells. Since spreading of cells is a regulator of growth and differentiation and transition metal ions like Co2+ have been shown to affect cell differentiation, too [2] such multilayer systems might be promising for making coatings on implants and scaffolds to regenerate tissues like bone, cartilage and others.

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Acknowledgements: This work was part of the High Performance Center Chemical and Biosystems Technology Halle/Leipzig and supported by the European Regional Development Fund (ERDF) and a grant from Martin Luther University Halle-Wittenberg for female PhD students in the final state of their project.

P13 Embedding novel cationic liposomes on the terminal layers of polyelectrolyte multilayer system to study the controlled release on myoblast cells

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Novel cationic liposomes made of OO4 (N-{6-amino-1-[N-(9Z) -octadec9-enylamino] -1-oxohexan-(2S) -2-yl} -N0- {2- [N, N-bis(2-aminoethyl) amino] ethyl} -2-hexadecylpropandiamide) and dioleoylphosphatidylethanolamine (DOPE) contain high amounts of amino groups and are suitable for lipofection. Such cationic liposomes might be also useful as a polycation in multilayer formation using Layer-by-Layer technique (LbL), which is a method to fabricate coatings by alternating adsorption of polyanions and polycations Since liposomes are suitable for controlled release of components to promote osteogenic differentiation for regeneration of bone, here, a polyelectrolyte multilayer system (PEM) was made of chondroitin sulphate (CS) and collagen type I (COL I) by LbL with OO4/DOPE liposomes embedded in the terminal layers. This work focused in physicochemical properties of PEM such layer growth, thickness, and topography using different methods of characterization. Also, we evaluated adhesion of C2C12 myoblast cells and cellular uptake of liposomes embedded onto PEM. The purpose of the PEM was to present the liposomes to C2C12 cells with internalization of lipophilic (Rhodamine-DOPE conjugate) and hydrophilic (Texas Red labeled dextran). COL and CS should mimic the extracellular matrix for future application such as an implantation in bone replacement therapies. Characterization was performed using ellipsometry and atomic force microscopy (AFM). The layer growth of PEM was studied using surface plasmon resonance (SPR). Cell adhesion and liposome uptake studies were carried out with fluorescence staining and flow cytometry analysis. SPR results shows a linear growth with an increase in the angle shift corresponding to the adsorbed mass of PEM. Liposome adsorption measured with elliposometry lead to a significant increase in thickness. AFM studies indicated that the final layer had a slightly rough morphology. Further, the adhesion studies showed a higher amount of cells on the terminal liposome layer. The quantification of liposome uptake indicated that the population of cells, where the liposomes were embedded into PEM took up 50.8% (Texas red) and 90.5% (Rhodamine). These results show the uptake efficiency of cationic liposomes embedded onto a PEM system for controlled release of compounds into the cells.

P14 Fabrication of antibacterial membranes with increased osteoconductivity for periodontal tissue regeneration

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INTRODUCTION: The presented solution addresses the issue of bone reconstruction in dentistry, in particular guided tissue regeneration (GTR) and guided bone regeneration (GBR). Both methods involve the restoration of bone deficiencies by means of barrier membranes. Thus, approach of both bone regeneration and soft tissues separation should be considered when designing new barrier membrane construct. In addition, in dental procedures the risk of bacterial infections is particularly high and antibacterial properties are highly demanded. Currently used membranes possess many structural, mechanical and bio-functional limitations [1]. The use of biodegradable polymers in a form of fibrous meshes fabricated via electrospinning method are good choice to overcome these limitations due to the flexibility in the use of the components and various morphologies. The introduction of nanohydroxyapatite (nHA) have been proved to enhance bone tissue regeneration. Therefore, electrospinning and application of nHA deposition on antibacterial fibers was proposed.
MATERIALS and METHODS: Fibrous meshes were prepared from PDLLA and PLGA polymers. The ZnO-based nanoparticles were used as a antibacterial agent mixed with polymer solution prior electrospinning. nHA particles were used for sonocoating of an implant according to the patented method [2]. It was proved that application of ceramic nanoparticles of high specific area can significantly increase water contact angle and cellular activity on implants [3]. ZnO-based antibacterial agent is expected to limit the bacterial colonization.
RESULTS: As a result of polymer blending with antibacterial agent and electrospinning solution uniform porous structure was created. The sonocoating process induced nearby the textile substrate resulted in depositing uniform nanohydroxyapatite layers of 150-200nm thickness. Scanning Electron Microscopy imaging revealed that nHA particles cover fibres but do not interfere with the structure of the base material. The porous structure of fibrous mesh was maintained practically unchanged and wettability of the material was highly increased.
DISCUSSION & CONCLUSIONS: The novel non-destructive method of nHA deposition on the fibres can serve as an alternative for currently used implant modification methods and enhance the bone regeneration process in bone/implant interface region. The introduction of a antibacterial agent was proved to be successful and expected to limit the bacterial colonization around the implant.

ACKNOWLEDGEMENTS: NANOLIGABOND (POIR.04.01.02-00-0016/16)

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P15 Antimicrobial multilayer coatings based on Collagen and Tannic acid

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Development of multifunctional materials is mandatory to challenge the complex processes like foreign body response, to cope with nosocomial infections and many more. Hospital acquired infections are caused by early attachment of bacteria during the surgery for example in case of medical implants' failure. The bacteria can grow into undesired slimy matrix called biofilms and its treatment becomes more difficult. Such fatal situations ultimately lead into an additional surgery, extra hospitalization time and increase in health care cost [1]. Therefore, methods to avoid early attachment and growth of bacteria gained huge popularity. Based on alternated deposition of oppositely charged polyelectrolytes [2], the layer-by-layer (LbL) technique attracts an increasing interest to design antimicrobial coatings due to its simplicity to implement and the control of the quantity adsorbed [3].

Herein, we successfully developed Tannic acid/Collagen (TA/COL) antimicrobial films using the layer-by-layer method. Buffer used to build TA/COL films is a pivotal parameter leading to different physico-chemical and biological properties. Built in acidic pH with two different buffers (citrate and acetate), TA/COL films have an exponential growing, observed by quartz crystal microbalance and a fibrillar or granular topography depending on the buffer. Fourier transformed Infrared spectroscopy and circular dichroism experiments showed that COL keeps its native structure in both buffers. Mainly stable up to 72 h in physiological medium, TA/COL films release TA in solution up to 25 µg/mL in PBS. A release-killing effect towards *Staphylococcus Aureus* was obtained only for films built in citrate buffer without cytotoxicity for potential host cell line like fibroblasts. Isothermal titration calorimetry measurements showed that the interaction between TA and COL are different in both buffers leading the immobilization of more TA along COL chains in the films. This work shows that the physico-chemical optimization of films containing polyphenols is of paramount importance for their future use as antimicrobial agents.

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P16 Bioabsorbable electrospun materials for *in situ* drug release in wound healing applications

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Cutting-edge biomaterials based on biomacromolecules are of growing interest in the field of biomedicine, especially for their application in wound healing. Materials are designed to improve therapy of secondary healing wounds or to cope with common complications like impaired healing or infection. Electrospinning is a widely used method for biomaterial fabrication, which allows the production of nano- and microfibrous nonwoven materials that can mimic the microstructure of the extracellular matrix. In order to address complications in wound healing, the embedding of drugs such as antibiotics or growth factors for its retarded release is a reasonable approach for the generation of innovative biomaterials. In this study, we designed a bioabsorbable fibrous nonwoven material for biomedical applications and examined the release of a drug analogue.

Polyethylene oxide (PEO) was dissolved at 3% (w/v) in 30% (v/v) acetic acid and fluorescein was added at a concentration of 1 mg/mL. Poly(L)-lactide and gelatin were dissolved in hexafluoroisopropanol and 50% (v/v) acetic acid at concentrations of 10% and 20 % (w/v), respectively. Coaxial electrospinning was performed on a Fluidnatek LE-50 device (Bioinicia, Valencia, Spain) using PLA or gelatin as sheath and PEO/fluorescein as core polymer. Gelatin-containing materials were stabilized by formaldehyde fumigation. The microstructure of the nonwoven materials was investigated by scanning electron microscopy and fluorescence microscopy. The release of fluorescein from the fibers was tracked by time-dependent UV/Vis spectroscopy under different conditions.

We successfully produced different fibrous materials by coaxial electrospinning and tracked the release kinetics of the drug analogue from the nonwoven materials. By adapting the constitution of the sheath polymer, we were able to alter the release kinetics. The here presented method allows the fabrication of bioabsorbable drug-loaded fibrous biomaterials with adjustable release kinetics for a wide range of biomedical applications, in particular cutting-edge wound dressings or implant coatings.

P17 A universal system to control the unspecific protein and cell adhesion on electrospun polycaprolactone wound dressings

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Electrospun polycaprolactone (PCL) fibers are attractive materials to fabricate wound dressings that mimic the native extracellular matrix, nonetheless surface bio-fouling of these structures when interfaced with biological media remains a severe issue. Undesired adhesion of proteins and cells can influence wound healing by triggering foreign body reaction and chronic infections. Preventing the nonspecific adsorption of proteins and adhesion of cells are key features for controlling the functionality of medical devices. In this poster, I will show a smart and multifunctional strategy to produce bio-fouling resistant electrospun PCL fibers. Herein, a bacterial killing mechanism based on amphiphilic antimicrobial peptides is turned into an advantage. The surface activity of the antimicrobial liquid chromatography peak I (LCI) peptide is exploited to achieve irreversible binding of a protein-polymer hybrid to the surface of PCL electrospun fibers via physical interactions and generate a repelling surface. The protein-polymer hybrid consists of two blocks, a surface-affine block (LCI) and a functional block that is equipped with antifouling polymers such as carboxybetaine methacrylamide (CBMAA) and *N*-hydroxypropyl methacrylamide (HPMA). The hybrids are synthesized in aqueous solution (PBS) by growing polymeric chains directly from the peptide using single electron transfer-living radical polymerization (SET-LRP). The electrospun PCL fibers are functionalized by simply immersing them into the polymerized hybrid solution. The binding density and protein-antifouling are analyzed by a combination of X-ray spectroscopy and surface plasmon (SPR) spectroscopy. The long-term stability of this coating system in contact with challenging culture media has been also evaluated. After 3 months, there was no detectable detachment nor microbial infiltration even in non-aseptic conditions. Remarkably the modified PCL surfaces were able to suppress non-specific protein adsorption from human blood plasma and minimized subsequent cell attachment.
We have developed a system that differs from current approaches because of its outstanding stability, ease and mild production aswell as its versatility. This establishes a universal method to tune the surface characteristic of electrospun scaffolds with a number of functional monomers without affecting their structure and enhancing target tissue regeneration.

Acknowledgement: The authors thank the support of the research association of Forschungskuratorium Textil e.V. supported via AiF ("Arbeitsgemeinschaft Industrielle Forschungsvereinigungen Otto von Guericke e.V."), research project IGF-No. 19893 N within the promotion program of "Industrielle Gemeinschaftsforschung" (IGF) of the Federal Ministry for Economic Affairs and Energy on the basic of a decision by the German Bundestag.

P18 Free-Standing Multilayer Films as Growth Factor Reservoirs for Wound Dressing Applications

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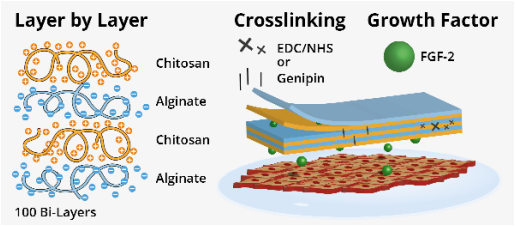
Introduction: Chronic skin wounds place a high burden on patients and health systems. The use of angiogenic and mitogenic growth factors could facilitate the healing process. Unfortunately, they are easily inactivated by the wound environment. We propose layer-by-layer (LbL) based growth factor releasing multilayer films as the basis for a novel wound dressing design.



Experimental methods: Free standing multilayer films were constructed by employing Chitosan (CHI) and Alginate (ALG) as opposing polyelectrolytes. 100 bilayers were applied and subsequently cross-linked to create a thick detachable film. Two cross-linking strategies were compared to the non-cross-linked films, using ethyl(dimethylaminopropyl) carbodiimide (EDC) combined with N-hydroxysuccinimide (NHS) and Genipin (GEN). Wetting, swelling, visco-elastic properties and crosslinking density were characterized. After crosslinking, fibroblast growth factor 2 (FGF2) was loaded into the films and its release was evaluated by ELISA. The films were added on top of normal human dermal fibroblasts (NHDFs). For these cells the metabolic cell activity, growth, migration and morphology was studied with the help of MTT and Qblue assays as well as immunohistochemical staining.

Results and discussions: EDC/NHS and Genipin cross-linked films showed improved mechanical strength, while retaining most of their swelling abilities necessary to take up wound exudates. Additionally, they showed sustained release of FGF2 was slower (following the Higuchi-model) which is beneficial in an oxidative wound environment. All films showed no cytotoxicity. Cell Proliferation and Migration was greatly improved by the cross-linked and FGF-2 loaded films. Genipin cross-linked film demonstrated a slight advantage over the EDC/NHS films. Interestingly the direct contact of films with the cells had a beneficial effect on cell growth compared to equal amounts of soluble FGF-2.

Conclusions: The results show that the proposed free standing multilayer films are able to act as a reservoir for the sustained local release of FGF2, which alleviates chronic wounds by stimulating granulation tissue formation. At the same time, they show properties that make them suitable as wound dressings by forming a physical barrier, showing mechanical resistance and swelling capabilities. This makes them a promising starting point for the design of active wound dressings.

P19 The artificial biofilm based on composite construction from bacterial poly(3-hydroxybutyrate) and alginate seeded with probiotic bacteria

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Nowadays, at surgery interventions on the intestine, polymer scaffolds are increasingly used to regenerate the tissues of the intestinal wall. But until recently, regenerative medicine of the gastrointestinal tract developed practically without consideration of the effect of intestinal microbiota on the regeneration of intestinal tissues that are densely inhabited by these bacteria. Whereas the bacteria of normal microbiota can contribute to the regeneration of intestine wall tissues and the composition of microbiota can be a marker for the process of wall tissue regeneration after the surgical patch implantation. Thus, the aim of this study was to develop a chimeric construction from bacterial biopolymers containing probiotic bacteria for tissue regeneration and to study the effect of the implantation of this construction on the local microbiota content in the large intestine of rats.

Poly(3-hydroxybutyrate) (PHB) and alginate were obtained by bacterial biosynthesis using producing strain *Azotobacter vinelandii* 12. Bacteria were cultivated in the liquid Burke medium with a minimum level of aeration for the synthesis of PHB, and with a maximum level of aeration for the synthesis of alginate. We developed the composite construction consisted of a PHB scaffold with the immobilized porous PHB microspheres. The probiotic bacteria *Lactobacillus plantarum* 8P-A3 and *Bifidobacterium longum* MC-42 were grown in the synthetized bacterial alginate as in a semi-wet medium. Then we filled the produced porous PHB-based scaffold with the bacterial alginate hydrogel seeded with the probiotic bacteria and without bacteria. The construct was implanted into Wistar rats in the site of colon wall defect. One week later, rats were euthanized and the samples of biomaterial were isolated from the site of construction implantation for metagenomic analysis. Metagenomic analysis was performed on the hypervariable region of V4 of the 16S rRNA gene.

The analysis of the obtained data showed that the bacteria from *Firmicutes* phylum dominates in preoperative and postoperative samples. The significant differences were found for three bacterial genera that appeared in the experimental samples: *Ileibacterium sp.*, *Lachnoclostridium sp.*, *Faecalibaculum sp.* A sharp decrease of *Erysipelatoclostridium sp* abundance was also found. Thus, the implantation of the PHB/alginate construction seeded with probiotic bacteria effects the local microbiota content in the large intestine of rats.

The work was supported by Russian Science Foundation, project # 17-74-20104.

P20 Thermoresponsive polyelectrolyte multilayers of chitosan and PNIPAm-modified cellulose sulfate for the generation of cell sheets for tissue engineering

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Thermoresponsive materials, as one kind of stimuli-responsive materials, used as surface modification for the generation of cell sheets have been in the focus of research for several years. Amongst other things, they allow the enzyme-free harvest of cells and the use of intact cell sheets, possessing extracellular matrix, for tissue engineering purposes. In this study, a thermoresponsive polyelectrolyte multilayer system that allows enzyme-free cell harvest is described. Consisting of polysaccharides, namely chitosan and cellulose sulfate, the multilayer is fabricated using a layer-by-layer coating technique. Cellulose sulfate is chemically modified with Poly(N-isopropylacrylamide) (PNIPAm) to achieve thermoresponsive behaviour. PNIPAm is one of the most famously known thermoresponsive polymers, applicable in a temperature range between 4 and 45 °C and hence within the limit of physiological conditions. Using the modified cellulose sulfate, thermoresponsive multilayer systems can be manufactured without the need of expensive equipment or radiation (e.g. in contrast to conventional methods, like electron beam irradiation). The physical properties of the polyelectrolyte multilayer have been characterized with several methods. Surface Plasmon Resonance (SPR) measurements, Quartz Cristal Microbalance and ellipsometry gave insight into the physical properties of the multilayer system, especially adsorption and thickness of the multilayer system. For biological characterization, cell adhesion (fibroblasts) has been tested. Furthermore, the detachment of those cells in dependence of the environmental temperature has been studied. Cells adsorb onto glass cover slips modified with a thermoresponsive multilayer system. After exposing it to environmental temperatures of 4 °C, cells successfully detach from these surfaces. The goal is to establish a multilayer system for cell harvest, which is easily manufactured and biocompatible.

P21 Development of biogenic thermoresponsive polyelectrolyte multilayers for the application on tissue engineering

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The stimuli-thermoresponsive polymer, Poly (N-isopropylacrylamide) (PNIPAM) exhibits a low critical solution temperature (LCST) at 32°C allowing cell adhesion at 37°C, while cell detachment can occur by simply reducing temperature below LCST. [1] The specific surface is commonly required for the modification of PNIPAM. Here, using layer-by-layer (LbL) technique to achieve the fixation of the thermoresponsive polymer as a culture substrate to harvest cell sheets is an effective method based on oppositely charged polyelectrolytes absorbed onto charged surface. [2] This thermoresponsive multilayers were constructed by chitosan covalently grafted with the thermoresponsive elements PNIPAM (PNIPAM-CHI) [3] as polycations and bioactive heparin as polyanions. The structures and the degrees of substitution (DS) of PNIPAM onto chitosan were analyzed by proton nuclear magnetic resonance (^{1}H NMR). Higher DS of PNIPAM-CHI becomes more gel-like solution and shows significant change in size compared to lower DS above LCST. The growth behaviors of multilayers built by all the different DS of PNIPAM-CHI and heparin had similar and linear growth tendency. There were 10-20° difference in water contact angle (WCA) between 37 °C and 4 °C in the PNIPAM-CHI system. For the preliminary cell study on thermoresponsive multilayer, fibroblasts could adhere well on the PNIPAM-CHI multilayers. The detachment process was performed by shifting cells from 37 °C to 4 °C. Cells were successfully detached from PNIPAM-CHI multilayers after 30 min detachment. The PNIPAM-CHI multilayers have the thermoresponsive effects on surface characterization as well as cellular response. This research will help to pave the way for development of bioactive glycosaminoglycans for cell harvesting and to generate cell sheets for tissue engineering and transplantation.

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Acknowledgements: This work is supported by International Graduate School AGRIPOLY funded by the European Regional Development Fund and the Federal State Saxony-Anhalt.

P22 Surface-Mediated Gene Delivery from functionalized Polyelectrolyte Multilayer Scaffolds in Tissue Engineering

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In recent decades, the development of therapeutic strategies in which the drug is a nucleic acid, is an important research focus in gene therapy. A major challenge for the establishment of gene therapy is still the overcoming of biological barriers by means of suitable delivery systems. A new strategy for nucleic acid delivery systems is the encapsulation or immobilization of gene vectors within biomaterial surfaces. This allows the DNA to be positioned in cellular microenvironment to achieve localized and efficient gene delivery to tissues or cells. In this study, we have developed a polyelectrolyte multilayer film (PEM-film) that permits both, the immobilization and contact-triggered release of DNA from the coated surface.

Our approach makes use of the layer-by-layer method for the assembly of nanostructured thin films consisting of alternating layers of hyaluronic acid and chitosan. Here, lipid/DNA complexes (lipoplexes), consisting of novel cationic lipids are embedded within PEMs. We focused on effective loading of the PEMs with DNA and on the intensive surface characterization using confocal fluorescence microscopy, ellipsometry, AFM and SEM. In addition, interactions with C2C12 myoblasts, e.g. cell adhesion and cell viability were investigated.

In summary, a system based on hyaluronic acid and chitosan could be produced which on the one hand can be loaded effectively with DNA and on the other hand can trigger localized surface-based transfection on C2C12 cells. Surface- und cell studies show that the PEM-scaffold is a nanostructured system which is capable of cell adhesion and shows very good cell viability. First in-vivo experiments with the chorion allantois membrane as animal replacement model were carried out in which a good transfection could also be achieved with our established system.

P23 Polysaccharide-Based Hybrid Microgel/Hydrogel Systems for Tissue Regeneration

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Polysaccharides, glycosaminoglycans and their derivatives are biocompatible and bioactive polymers that can be harvested from regenerative sources and used as building blocks of scaffolds and hydrogels in the field of tissue engineering and regenerative medicine. Oxidized sodium hyaluronate (oxHA), which contains aldehyde groups and carboxymethylchitosan (CMC), which contains amine groups, are used to function as precursors to the hydrogel. The amine and the aldehyde groups react to form reversible imine bonds so that cell laden stable hydrogels can be synthesized and used as a scaffold without the need of a potentially toxic crosslinker. Under physiological conditions, these hydrogels can eventually degrade, while being slowly replaced by growing cells. Different oxidation degrees of the oxHA and different ratios of oxHA:CMC lead to hydrogels with different properties and degradation times. Tests of the sodium hyaluronate show a lower biocompatibility in samples with higher degrees of oxidation.

During incubation, it was found that some hydrogels show a very fast degradation. To further enhance the stability and longevity of the hydrogels, particles composed of sodium hyaluronate derivatives can be incorporated during the gel formation process through additional crosslinking. The particles are composed of oxHA and hydrazide functionalized HA which, when mixed in an inversed emulsion droplet can crosslink to form stable hydrazone bonds. After the particle formation the surface is decorated with unreacted aldehyde groups, which can further crosslink with the CMC. Variations of the microgel synthesis were explored to investigate the influence on the particle size.

By encapsulating growth factors or enzymes in the particles, a burst release of the compound into the surrounding tissue, which normally occurs, when conventional hydrogels are loaded with such compounds can be prevented and the drug release rate becomes more constant. The particle cytotoxicity shows an increased biocompatibility in comparison to the oxHA compound of the hydrogel while the microgel/hydrogel hybrid systems show an increased stability in comparison to conventional hydrogels.

The synthesized hydrogels can be potentially used for the engineering of cartilage tissue.

P24 Human hepatocytes encapsulated in injectable hydrogels of hyaluronic acid and gelatin

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The only curative treatment for severe end-stage liver disease is liver transplantation. However, it is limited by the shortage of organ donors. The increase of the incidence of liver diseases has led to develop new therapeutic approaches such as liver cell transplantation. Cell-based liver therapy is envisaged as a potentially useful therapeutic option to recover and stabilise the lost metabolic function in different liver diseases to ameliorate the clinical outcome. However, transplanted hepatocytes cannot grant the best clinical results due to their low engraftment efficiency and survival.

The main objective of this research is to improve the clinical results of liver cell transplantation by the use of strategies which optimise both delivery and retention of the cells in the host liver and which mimic cell-cell and cell-matrix interactions *in vivo*. We used natural hydrogels based on the components of the extracellular matrix, hyaluronic acid (HA) and gelatin (Gel), to better mimic the natural environment of cells. Hydrogels were crosslinked through enzymatic reaction by hydrogen peroxide and peroxidase.

For this purpose, we evaluated the benefits of using injectable hydrogels to encapsulate human hepatocytes for their use in liver cell therapy. Hydrogels were made of 100% HA, 100% Gel and a mixture containing 80% HA and 20% Gel. Results have shown that the best material was the mixture HA-Gel, as cell viability and ureogenesis was optimal. Basic hepatic functions such as ureogenesis, cytocrome P450 activity and albumin secretion of human hepatocytes encapsulated within the selected hydrogel were improved by 3D environments in comparison with hepatocytes cultured in monolayer. We conclude that bio-inspired injectable hydrogels are suitable to perform hepatic cell culture and thereby promising candidates for liver cell-based therapies.

P25 Generation of mature hiPSC derived hepatocytes: a nature-inspired protocol

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The production of hepatocytes derived from human induced pluripotent stem cells (hiPSC) holds great promise not only for in vitro disease modelling and drug screening applications but also for the regenerative medicine field through the possibility of producing bioartificial livers. Nonetheless, generated hepatocytes are still immature when compared to the adult counterparts. The major hurdle in recapitulating in vitro the physiological liver maturation process is due to its complexity as it takes approximately 2 years after birth and involves the induction of a wide range of metabolic and detoxification pathways.

Recent findings have been suggesting that liver maturation and functionality, that naturally occur during the early postnatal period, could be strongly associated with gut microbiome (1).

In this work, we developed a nature-inspired protocol to generate more mature hepatocytes from hPSC by evaluating the impact of 3D culture strategies and human gut microbiome on hepatocyte differentiation yields and purity.

hPSC were differentiated into hepatocyte-like cells (HLC) using either a conventional monolayer (2D culture) or a scalable protocol based on the cultivation of cell as 3D aggregates in stirred-tank bioreactors operated in perfusion. At day 21 of differentiation, HLC were treated for additional 6 days with intestinal bacteria secretome generated from neonatal or adult samples.

Our results showed that, HLC generated in 3D culture exhibited higher expression of some hallmarks of mature liver (CYP3A4, HNF4a and ALB), an increase in the albumin production, higher uptake and release of cardiogreen and glycogen storage when compared with the HLC generated in 2D monolayers. Treatment with gut microbiome secretome induced the gene expression levels of CYP3A4, CYP2C9, HNF4a and ALB in HLC which was not verified in cells treated with culture medium only or supplemented with secretome from a donor under antibiotic treatment. Interestingly, the secretome from neonatal origin proved to be more efficient in driving hiPSC hepatic maturation than the adult formulation.

In conclusion, our work provides, for the first time, novel insights into the interaction of human gut microbiome secretome and maturation of hiPSC-derived hepatocytes. The protocol developed herein for the production of hiPSC-hepatocytes presents high technological relevance due to its efficiency, scalability and reproducibility. These will strength the use of these cells in regenerative medicine and pre-clinical research.

Acknowledgements & Funding

Joana Almeida and Pedro Vicente acknowledge *Fundação para a Ciência e Tecnologia* (FCT) for the PhD fellowships SFRH/BD/116780/2016 and SFRH/BD/145767/2019, respectively. This work was supported by Project EHD16PI02 from CIBERehd and Project LMP226_18 funded by DGA, Zaragoza, Spain as well as by FCT-funded projects ERAdicatPH (E-Rare3/0002/2015), and iNOVA4Health Research Unit (LISBOA-01-0145-FEDER-007344), which is cofunded by FCT/MCES, through national funds, and by FEDER under the PT2020 Partnership Agreement.

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P26 Efficiency of mononuclear stem cells and statinotherapy in rats with non-alcoholic steatohepatitis

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Excessive accumulation of lipids by hepatocytes, progression of lipid peroxidation, activation of apoptosis lead to development of nonalcoholic steatohepatitis (NASH).

Objective: To study the effect of mononuclear stem cells (MSCs) and statin on the state of liver in rats with NASH.

Materials. Studies were performed on 80 Wistar rats. NASH was simulated by atherogenic diet with the addition of 50 g / kg of pig fat for 90 days. Animals were divided into groups: 1st - control (n = 10) - NASH rats; 2nd (n = 30) rats with NASH and intragastric administration of rosuvastatin; 3rd (n = 30) rats with NASH and introduction of MSCs into liver at 7th and 14th days; intact group (n = 10). Animals were removed at the 30th day after the start of correction. MSCs were isolated by bone marrow sedimentation on Gelofusine.

Results. On the 90th day NASH was formed (alanine aminotransferase (ALT) activity was 167 ± 7 U/l, aspartate aminotransferase (AST) – 124 ± 4 U/l, low-density lipids (LDL) level – 2.01 ± 0.38 mmol/l, high-density lipids (HDL) – 0.38 ± 0.08 mmol/l, triglycerides (TG) – 1.56 ± 0.6 mmol/l; protein dystrophy, foci of necrosis, steatohepatosis grade III-IV).

On the 30th day after correction in group I revealed: ALT activity 155 ± 15 U/l, AST – 115 ± 4 U/l, LDL level – 1.9 ± 0.38 mmol/l, HDL – 0.41 ± 0.07 mmol/l. In group II compared to group I ($p < 0.04$) ALT activity was 21 % decreased, AST – 15 % decreased, LDL level – 40 % decreased, TG – 16 % dereased, and HDL level was 20 % increased. In group III compared to group I ($p < 0.04$), ALT activity was 47 % decreased, AST – 25 % decreased, LDL level – 47 % decreased, TG – 22 % decreased, HDL level was 23 % increased. In group I, the liver had pronounced protein dystrophy, damaged cytoarchitectonics, foci of necrosis, diffuse fatty hepatosis of grade III-IV; in group II - protein dystrophy, intracellular bilirubinostasis, histiocytic infiltration and foci of necrosis, diffuse fatty dystrophy of grade III-IV; in group III - protein dystrophy, cholestasis, microvesicular fatty dystrophy grade II.

Conclusions. Statinotherapy led to positive changes in the lipidogram, but not the structure of the liver. Administration of MSCs into liver led to improvement of lipidogram, decreased hepatic transaminases activity, improved liver regeneration.

P27 Development of a bio-artificial kidney as a cell-based renal model

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A requirement for the development of a new drug is prediction of human pharmacokinetics and drug-drug interactions (DDIs). This may include the prediction of interactions with transporters expressed in the kidney. Currently, these characterizations are based on 2D cellular assays, however in vitro- in vivo discrepancy may be improved by mimicking in vivo conditions of renal cells. Bioartificial kidney (BAK) devices consist of a porous polymer hollow fibres (HF) possibly coated by extracellular matrix (ECM) to support renal cells attachment and growth, while a steady flow of media supplies nutrients and removes waste. The porous nature of these fibres will allow small molecules to cross and these can be actively transported by renal cells expressing renal transporters. The aim of this project is to develop a BAK device with a biocompatible HF to be used as an accurate renal model.

Several biomaterials were produced and tested for their ability to provide an adequate scaffold for cell adhesion while retaining their function. This was tested by culturing human embryonic kidney (HEK) cell expressing organic cation (OCT2) transporter in a 2D platform on three porous membranes, Polysulfone (PSF), Polyethersulfone (PES) and Polyvinylidene fluoride (PVDF). PVDF based biomaterials have shown to have ideal cell adhesion properties while cells were retained their substrate uptake activity which remained unaltered, compared to cells cultured on tissue culture plastic.

PVDF based HFs were then attempted to be produced and although they have retained their cell adhesion properties, they lacked consistency and porosity, making them unsuitable for a BAK device. Therefore, a commercially available Polypropylene based HF (Plasmaphan P1LX, 3M) was used instead. Although it had good cell adhesion properties using both HEK-OCT2 and MDCK-MDR1a cell lines, this was enhanced with the use of renal relevant coating Geltrex containing the base matrix proteins Laminin, Collagen IV, Enactin and Heparin sulfate proteoglycans. A BAK device that could house a single HF with cells seeded in the lumen was then developed to mimic renal physiology. The HF lumen mimics the renal proximal tubule while the Extracapillary space (ECS) mimics the peritubular capillaries. In addition, a constant flow of media on both Lumen and ECS mimics the ultrafiltrate and blood flow respectively, while exposing cells to shear stress.

Results have shown that shown that MDCK cells overexpressing the transporter P-gp (MDCK-P-gp) were seeded in the HF lumen form a monolayer when exposed to shear stress. In addition, qPCR results have shown significant changes in gene expression compared to cells grown in 2D. Basolateral markers such as Na/K ATPase, transporter P-gp and tight junction marker ZO-1 have shown a twofold expression reduction compared to cells grown in static conditions, while the proliferation marker Ki-67 and the microvilli marker CD133 have shown a twofold increase.

These results indicate that the BAK device influences cultured cells in comparison to conventional cell cultures. Due to the 3D environment and shear stress, we hypothesise that cells have a more physiological phenotype than cells grown in 2D.

The next step would be to study cell function using efflux/influx assays using fluorescent substrates in the BAK device and compare this with 2D cell cultures. The final step would be the utilisation of human primary renal cells in a pharmaceutical screening set up to investigate the potential use of the BAK device as a model in drug discovery.

P28 Adaptive *de novo* fibrinolytic hemocompatible membranes for artificial lung assists

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The membranes of current artificial lung assists lack sufficient hemocompatibility, which leads to unwanted activation of hemostasis and the need of high doses of anticoagulants that result in potential life-threatening complications. To overcome this challenge we developed a new concept for coatings that combines passive properties (prevention of protein adsorption and activation of coagulation) with active ones (disintegration of thrombi formed upstream of the membrane) to enhance the hemocompatibility of the membranes. This was accomplished by immobilizing tissue plasminogen activator (tPA) on poly(*N*-2-hydroxypropyl methacrylamide-*co*-carboxybetaine methacrylamide) (poly(HPMA-*co*-CBMAA)) brushes grafted from poly(4-methyl-1-pentene) (PMP) membranes. The immobilized enzyme simultaneously captures the fibrin network of a thrombus and plasminogen, which is then activated to plasmin that digest the trapped thrombus. Here we explore a switchable attraction between tPA and the brushes, which is followed by covalent binding of 76.9 $ng \cdot cm^{-2}$ tPA on brushes grafted from PMP membranes. The activity of tPA was proved by chromogenic substrates that mimic plasminogen and fibrin, respectively. We incubated the modified membrane with a solution of plasminogen in buffer and a fibrin-mimetic chromogenic substrate in physiological conditions. Only 15 min were sufficient to completely digest the substrate, highlighting the very high activity of our coatings. The enhanced hemocompatibility of our coating by stopping coagulation and digesting thrombus was assessed in static and dynamic blood experiments. In the static experiments a drop of blood was placed on the membrane, while to mimic circulation we utilized a dynamic Chandler loop model. In both cases blood was slightly heparinized. This is necessary because the contact with air inevitably activates coagulation. The formation of thrombus was rapidly observed on the uncoated PMP surface in static experiments and SEM microscopy revealed the strong adhesion of platelets and fibrin. By contrast the thrombus formation was delayed on PMP membranes with brushes and did not attached to them. The onset of the thrombus formation was observed after 1 h. However, immobilized tPA was able to dissolve it and after 2 h no thrombus could be observed, nor any fibrin or platelets adhered. These results demonstrate that the combination of passive repellency with adaptive fibrinolysis is a powerful tool to counteract thrombus formation.

Acknowledgement. The authors thank the support of the research association of Forschungskuratorium Textil e.V. supported via AiF (“Arbeitsgemeinschaft Industrielle Forschungsvereinigungen Otto von Guericke e.V.”), research project IGF-No. 19893 N within the promotion program of “Industrielle Gemeinschaftsforschung” (IGF) of the Federal Ministry for Economic Affairs and Energy on the basic of a decision by the German Bundestag.

P29 Covalent immobilization and multilayer formation of glycosaminoglycans and their anti-inflammatory mechanism of action

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Biomaterial implants are targeted to improve the patient's quality of life. However, chronic inflammation and subsequent fibrotic encapsulation that can occur after implantation of biomaterials are issues that fostered efforts in designing novel biocompatible materials to modulate the immune response. Inflammation is orchestrated by macrophages through the release of cytokines, which synthesis is dependent on nuclear transcription factor-κB (NF-κB) signaling pathways. Glycosaminoglycans (GAG) like heparin (Hep) and high molecular weight hyaluronic acid (HA) are known to have an effect of NF-κB signaling. Here, we studied the biomaterial´s coating either by covalent immobilisation as monolayer or by assembling polyelectrolyte multilayers (PEM) together with chitosan (Chi) through layer-by-layer (LbL) technique on model substrata that were characterized by different physical methods. The fruitful physicochemical characterization allow us to investigate further the effect of Hep and HA on inflammatory response of THP-1 derived macrophages. Adhesion, interleukin I release and formation of multinucleated giant cells (MNGCs) found for both systems (covalent vs. LbL) an inhibition compared to controls, with higher efficiency of Hep in multilayer systems. Investigation of signal transduction was done through immunofluorescence staining of p65 subunit of NF-κB to estimate the nuclear to cytoplasmic ratio, which was indicating a reduced translocation of p65 to the nucleus in cells plated on GAG-modified surfaces. Studies with western blotting of p65 from cell extracts showed also reduced expression in terms of the phosphorylated and non-phosphorylated forms of NF-κB that are related to an inhibition of macrophage activation by GAG. Association and endocytosis of FITC-labelled GAG assay were evaluated by confocal laser scanning microscopy and flow cytometry showing that macrophages were positive toward the uptake of immobilised GAG either by association or endocytosis of HA and Hep. These results illustrate that the anti-inflammatory activity of GAG is not only related to making surfaces more hydrophilic which reduces protein adsorption and macrophage adhesion, but also their active involvement in signal transduction processes related to inflammatory reactions, which makes such coatings interesting for implantable biomedical devices.

P30 Immunological Potential of Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs) are the precursor cells of the connective tissue and can differentiate into distinct lineage specific cells such as chondrocytes, myocytes, adipocytes and osteoblasts. They can contribute to regeneration by migration to injured sites and integrate actively into the tissue, thereby counteracting degeneration. MSCs can also release cytokines, chemokines or hormones into the target site and MSCs have the potential to modulate the innate and acquired immune response. MSCs can be isolated from a number of tissues, particularly bone marrow, adipose tissue or placenta and can be expanded ex vivo. The mechanobiological behavior of cultured MSCs is based on the development of actin filaments which occur as stress fibers and on mitochondrial dynamics necessary for the energy metabolism.

Here we examined whether human platelet lysate (HPL), that can potentially replace fetal bovine serum (FBS) supplementation for clinical-scale expansion of functional MSCs, can modulate stress fiber formation, alter mitochondrial morphology, change membrane elasticity as well as motility and modulate the immune regulatory molecules. The culture supplementation with HPL showed a reduction of the actin concentration and related stress fibers in adhered two-dimensional (2D) cultured MSCs compared to conventional growth medium which contains FBS. Adhered 2D MSCs had no impact on membrane elasticity when propagated in the presence of HPL. The mitochondrial mass of adhered 2D MSCs was reduced by HPL, however, it was initially low in three-dimensional (3D) MSC spheroids, furthermore an increase in punctate and networks mitochondria was observed in adhered 2D MSCs of passage 3. Finally, the stimulation with pro-inflammatory cytokines IFN-γ and TNF-α showed gene and protein expression of the immunomodulatory molecule IDO, the supplementation with HPL showed no effect. GARP, in contrast, was constitutively expressed with no response to supplementation with HPL or stimulation with IFN-γ and TNF-α.

In conclusion, we can say that MSCs are sensitive to cultivation conditions in 2D and 3D with changes in their molecular composition that can have an impact on the immunomodulatory function.

P31 Functional capacities of extracellular vesicle micro-RNA in autologous blood-derived products

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Autologous blood-derived products have been applied in osteoarthritis therapy for over a decade, however, the mechanisms of action still remain unclear. Traditionally, research has focused on growth factors present in blood products to explain clinical outcomes, but in recent years, there is growing evidence that extracellular vesicles (EVs) are genuinely responsible for the regenerative potential of blood-derived products. EVs are nano-sized membrane particles of around 50-800nm that are generated by a plethora of cells and are found in all body fluids. They contain signal molecules such as micro RNA (miRNA), shuttle these molecules between cells and can regulate gene expression post-transcriptionally. The aim of the study was to determine miRNA profiles in purified EVs from plasma- and serum-based blood products to understand the functional capacities of miRNAs in EVs with respect to cartilage homeostasis, regeneration and inflammation. EVs were purified from citrate anti-coagulated platelet rich plasma (CPRP) and hyperacute serum (hypACT) via ultracentrifugation and size-exclusion chromatography. Particle concentrations and sizes were assessed via nanoparticle tracking analysis (NTA). Presence of EVs was confirmed via cryo-electron microscopy. EV miRNAs were analysed by screening a 372 primer panel via RT-qPCR. EV concentration was lower in hypACT than CPRP, while particle mode sizes were similar. Similarly, the miRNA diversity was lower in hypACT than CPRP. The miRNA populations shared 90% of individiual miRNA species between blood products, but only 30% between EVs. Relative miRNA abundances correlated in CPRP blood product and EVs, but show strong enrichment and depletion of individual miRNAs in hypACT. The miRNA profile enriched in hypACT EVs was functionally associated with inhbition of cartilage degradation, inhibition of inflammation, reduction of senescence as well as promotion of cell proliferation while CPRP EVs contained miRNAs with pro- and anti-chondrogenic miRNAs. The characterised miRNA profiles of purified EVs from CPRP and hypACT were very distinct in terms of quality and quantity. This not only adds another dimension of complexity in understanding the mechanisms of action of blood products, but also extends knowledge about their functional capacities.

P32 Use of Size-Exclusion Chromatography for the Removal of Co-Enriched Factors from Platelet-Derived EVs

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Introduction: EVs are cell-derived nanoscale vesicles that carry bioactive compounds derived from their cells of origin. The enrichment of EVs void of co-isolated contaminants is limited by their heterogeneous nature and overlapping size and density with other biological structures. Our previous experiments showed that high levels of the plasma protein alpha-2-Macroglobulin (α2M, 720kDa) were co-enriched with platelet-derived EVs at 100,000g centrifugation.
The aim of this study was to compare size exclusion chromatography (SEC) columns (Izon Science, Christchurch, New Zealand), with a pore size cut off of 70nm (qEVoriginal, 5mL) and 35nm (qEVsingle, 1mL) to deplete major co-isolated factors from EVs.
Methods: EVs were enriched from medical grade platelet concentrates by differential centrifugation at 20,000g (20k EV) and 100,000g (100k EV). The pellets were resuspended in PBS, adjusted to a protein concentration of 1µg/µL, and 100µL and 500µL, respectively, were loaded onto 1mL and 5 mL columns. Fractions were collected (1mL column: 200µL, 5mL column: 500µL) and the protein content was determined using DC Protein Assay (Bio-Rad, CA). Particle counts were measured using Nanoparticle Tracking Analysis (NTA, ZetaView, Particle Metrix, Inning, Germany). Fractions with overlapping protein content and EV counts were pooled. The presence of α2M was assessed by Western Blotting (0.5µg total protein/lane). NTA was performed in scatter mode and in fluorescent mode with the membrane stain CellMask Orange. EVs were characterized by flow cytometry (CytoFLEX LX, Beckman Coulter, CA) with lactadherin (LA) as marker for phosphatidylserine and CD41 as platelet marker.
Results: A complete depletion of α2M was possible using qEVoriginal column, while the purification with qEVsingle column only allowed for its partial removal.
Characterization of EV fractions by NTA and flow cytometry is summarized in the table below.

SEC column	EV fraction	NTA						Flow cytometry	
		Mean particle size [nm]		Particles/mL in scatter mode		%CMO$^+$ of all particles		%CD41$^+$LA$^+$ of all events in the EV gate	
		Before SEC	After SEC	Before SEC	After SEC	Before SEC	After SEC	Before SEC	After SEC
qEVoriginal	20k EV	169	170	1.1 x 10^{12}	1 x 10^{11}	17	33	51	63
	100k EV	167	145	8 x 10^{11}	9 x 10^{10}	20	25	12	n.d*
qEVsingle	20k EV	181	177	9 x 10^{10}	1.4 x 10^{10}	13.4	52	43	1
	100k EV	180	157	8.3 x 10^{10}	2.1 x 10^{10}	14.5	37.7	19	18

* not detectable

Conclusion: SEC purification by qEVoriginal column (cutoff 70nm) can more efficiently deplete α2M contamination when compared to the qEVsingle column (cutoff 35nm). We are currently testing whether SEC-based purification of EVs leads to increased co-isolation of lipoproteins.

Acknowledgement: Work funded by NÖ Forschungs- und Bildungsges.m.b.H. (NFB) and provincial government of Lower Austria. Life Science Calls (Project ID: LS16-018).

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