Hydrogels for light delivery in a biohybrid implant

Sonja Johannsmeier, Industrial and Biomedical Optics Department, Laser Zentrum Hannover e.V., Germany, and Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (NIFE), Hannover, Germany, s.johannsmeier@lzh.de
Maria Leilani Torres-Mapa[a,b], Tammo Ripken[b,c], Dag Heinemann[b,c], Alexander Heisterkamp[a,b]; [a]Institute of Quantum Optics, Gottfried Wilhelm Leibniz Universität Hannover, Germany; [b]Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (NIFE), Hannover, Germany; [c]Industrial and Biomedical Optics Department, Laser Zentrum Hannover e.V., Germany; torres@iqo.uni-hannover.de, t.ripken@lzh.de, d.heinemann@lzh.de, heisterkamp@iqo.uni-hannover.de

Light-based therapies have been established for various indications, such as skin conditions, cancer or neonatal jaundice. Advances in the field of optogenetics open up new horizons for light-tissue interactions with an organism-wide impact. Excitable tissues, such as nerve and muscle tissues, can be controlled by light after the introduction of light-sensitive ion channels. Since these organs are generally not easily accessible to illumination *in vivo*, there is an increasing need for effective biocompatible waveguides for light delivery. These devices not only have to guide and distribute the light as desired with minimal losses, they should also mimic the mechanical properties of the surrounding tissue to ensure compatibility. In this project, we are tuning both optical and mechanical properties of hydrogels from poly(ethylene glycol) derivatives to achieve compatibility with cardiac muscle tissue as well as optimal light guiding and distribution for optogenetic applications in the heart. The excitation light is coupled into the hydrogel with a biocompatible fiber. Properties of the hydrogel are mainly tuned by monomer length and concentration. Total reflection can be achieved by embedding a fiber-shaped hydrogel with a high refractive index into a second, low refractive index gel. Multi-component gels and different geometries are explored as possibilities for waveguiding. Scattering microparticles enhance light distribution throughout the hydrogel for an even distribution in the target tissue. After optimization, the hydrogel may be used to deliver light to various tissues for optogenetics applications or phototherapy.
The hydrogel waveguide is biocompatible and can be engrafted onto the target tissue. We are developing a biohybrid device encorporating a hydrogel for light delivery and light-sensitive engineered cardiac tissue for implantation to treat cardiac arrhythmias. The cardiac tissue patches are produced from induced pluripotent stem cell-derived cardiomyocytes and could be made from the patient's own cells in a clinical setting. This project evaluates the clinical relevance of optogenetics in cardiology and explores difficulties and opportunities for novel light-based therapies.

Cultivation and Differentiation of Respiratory Epithelial Cells on Polycarbonate Urethane Nonwovens

Anja E. Luengen, Department of Biohybrid & Medical Textiles, AME-Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany and AMIBM-Maastricht University, Geleen, The Netherlands, anja.luengen@rwth-aachen.de
Sophie Bezela, Department of Cardiovascular Engineering, AME-Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany, bezela@ame.rwth-aachen.de
Caroline Kniebs, Department of Biohybrid & Medical Textiles, AME-Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany and AMIBM-Maastricht University, Geleen, The Netherlands, kniebs@ame.rwth-aachen.de
Christian G. Cornelissen, Department of Internal Medicine I, Section for Pneumology, Uniklinik RWTH Aachen and Department of Biohybrid & Medical Textiles, AME-Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany and AMIBM-Maastricht University, Geleen, The Netherlands, ccornelissen@ukaachen.de
Stefan Jockenhoevel, Department of Biohybrid & Medical Textiles, AME-Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany and AMIBM-Maastricht University, Geleen, The Netherlands, jockenhoevel@ame.rwth-aachen.de
Anja Lena Thiebes, Department of Biohybrid & Medical Textiles, AME-Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany and AMIBM-Maastricht University, Geleen, The Netherlands, thiebes@ame.rwth-aachen.de

As part of regenerative medicine, tissue engineering combines living cells and bioengineered, artificial scaffolds to assemble them into functional constructs for restoration, replacement or maintenance of damaged or diseased tissues or organs. In previous studies, a biohybrid airway stent (PulmoStent) has been developed to treat stenoses caused by bronchotracheal cancer. It combines a handbraided Nitinol stent with a polycarbonate urethane (PCU)-based nonwoven to avoid tumor ingrowth. In order to maintain the mucus transport throughout the whole luminal stent surface, the aim of this study was adhesion of functional respiratory epithelial cells on the polycarbonate urethane layer. The pulmonary epithelial tumor cell line A549 was used for preliminary experiments, primary respiratory epithelial cells for differentiation studies.
The used PCU nonwovens dissolve in most standard histology solvents (e. g. xylene) and require special care during fixation, embedding, and cutting. Therefore, we developed a tailored sample preparation process in combination with an immunohistochemical staining protocol to prove epithelial cell differentiation. Furthermore, we employed a collagen IV-based coating procedure to improve cell adhesion and formation of an epithelial cell monolayer. In preliminary experiments, cells were seeded on (precoated) PCU nonwoven and cultivated for three days. Histologically, we could prove that the collagen-IV-coating favorably influences the cell adhesion and the microtome cutting properties. For the examination of epithelial cell differentiation on collagen-coated PCU, primary cells were cultured in a liquid-liquid-interface on precoated PCU and inserts (Corning Transwell) as a positive control for one week to achieve confluence. Afterwards, cell differentiation was triggered in air-liquid-interface for three weeks. Cilia formation was analyzed by immunohistochemistry and transmission electron microscopy. Ciliated epithelial cells on the luminal side of the stent can guarantee an improved mucociliary clearance *in vivo*.

Off-the-shelf vessel substitutes based on elastin-like recombinamers

Alicia Fernández-Colino, Dept. of Biohybrid & Medical Textiles, RWTH-Aachen University, Aachen, Germany, fernandez@ame.rwth-aachen.de
Frederic Wolf, Dept. of Biohybrid & Medical Textiles, RWTH-Aachen University, Aachen, Germany. wolf@ame.rwth-aachen.de
Hans Keijdener, Dept. of Biohybrid & Medical Textiles, RWTH-Aachen University, Aachen, Germany keijdener@ame.rwth-aachen.de
Thomas Schmitz-Rode, Institute of Applied Medical Engineering, Helmholtz Institute, RWTH-Aachen University, Aachen, Germany
smiro@ame.rwth-aachen.de
Stefan Jockenhoevel, Dept. of Biohybrid & Medical Textiles, RWTH-Aachen University, Aachen, Germany & Aachen-Maastricht-Institute for Biobased Materials (AMIBM) Maastricht University, Geleen, The Netherlands jockenhoevel@ame.rwth-aachen.de
José Carlos Rodríguez-Cabello, G.I.R. Bioforge, University of Valladolid, CIBER-BBN, Valladolid, Spain roca@bioforge.uva.es
Petra Mela, Dept. of Biohybrid & Medical Textiles, RWTH-Aachen University, Aachen, Germany mela@ame.rwth-aachen.de

During 2016, 50,114 coronary artery bypass grafting were performed in Germany (Beckmann, 2017). This high incidence has motivated a great deal of research to engineer vascular substitutes. Current efforts are focused on the development of off-the shelf vascular grafts able to support tissue regeneration *in situ*, i.e. upon implantation in the body. When compared to the classical tissue engineering strategies, an *in situ* approach entails multiple advantages: i) ready availability, ii) easy storage and transport and iii) faster clinical adoption.
In this work, we developed macroporous vascular grafts for *in situ* tissue engineering by using Elastin-Like Recombinamers (ELR), a novel class of recombinant biomaterials which feature outstanding properties such as elastic mechanical performance, tailored bioactivity, biocompatibility and hemocompatibility (De Torre, 2015). The vascular grafts were fabricated by the injection molding technique and subsequent crosslinking via catalyst-free click-chemistry. Salt-leaching/gas foaming strategies were used to create the porosity (Fernández-Colino, 2018). The resulting vascular grafts displayed a controlled porosity which could be reproducibly tuned, as visualized by SEM and two-photon microscopy. Importantly, cell infiltration into the inner areas of the graft was evident, with a concomitant ECM production, as visualized by SEM and corroborated by immunohistochemistry. Furthermore, these grafts were proved to withstand the physiological pressure conditions, and they were suturable. Overall, these results show the potential of the developed vessel substitutes for *in situ* tissue engineering.

Optogenetic modulation of cardiac activity using spatial controlled light patterns

Laura Diaz-Maue, Max Planck Institute for Dynamics and Self-organisation, Biomedical Physics Research Group, Goettingen, Germany, laura.diaz@ds.mpg.de
Stefan Luther, Max Planck Institute for Dynamics and Self-organisation, Biomedical Physics Research Group, Goettingen, Germany, Department of Pharmacology and Toxicology, University Medical Center, Goettingen, Germany
Institute for Nonlinear Dynamics, Georg-August-University Goettingen, Germany, German Center for Cardiovascular Research, DZHK partnersite Goettingen, Germany, stefan.luther@ds.mpg.de
Claudia Richter, Max Planck Institute for Dynamics and Self-organisation, Biomedical Physics Research Group, Goettingen, Germany, Department of Cardiology and Pneumology, University Medical Center, Goettingen, Germany, German Center for Cardiovascular Research, DZHK partnersite Goettingen, Germany, claudia.richter@ds.mpg.de

Cardiac arrhythmias are a life-threatening condition which might lead to death if left untreated. Conventional therapy methods including cardiac pacing, resynchronisation therapy and defibrillation, rely on electric fields which are applied directly to the heart and can provoke myocardial injury. Hence alternative techniques for restoring the normal cardiac activity need to be investigated. Lately optogenetics became one powerful tool that can be used to better understand the complex mechanisms underlying cardiac arrhythmias, thereby opening a new path towards the development of novel heart protective treatments. In this work we established an experimental setup to study the spatiotemporal behaviour of transgenic murine hearts by means of optical mapping. Furthermore we implemented an advanced photostimulation platform utilizing a Digital Micromirror Device, which is able to generate high speed and high spatial resolution digital light patterns. This special kind of spatial light modulation is designed for fine steering the epicardial surface and thus for the gentle modulation of cardiac activity. Our experimental results show that the successful induction and termination of cardiac arrhythmias using a non-invasive multi-site photostimulation approach can be achieved. Therefore demonstrating the suitability of the experimental setup for investigating cardiac phenomena and its potential application for developing alternative photostimulation therapies which might be translated in a future to other mammalian models.

The BioPacer, a tissue-engineered solution for atrioventricular-block.

Hans Keijdener, Dept. of Biohybrid & Medical Textiles RWTH-Aachen, Aachen, Germany, keijdener@ame.rwth-aachen.de
Rudolf Degen, Dept. of Chemosensation, Biology II, RWTH Aachen University, Aachen, Germany, r.degen@sensorik.rwth-aachen.de
Thomas Kuenzel, Dept. of Zoology/Animal Physiology, Biology II, RWTH Aachen University, Aachen, Germany, kuenzel@bio2.rwth-aachen.de
Bernd Hoffmann, Institute of Complex Systems 7: Biomechanics, Forschungszentrum Julich, Julich, NRW, Germany, b.hoffmann@fz-juelich.de
Rudolf Merkel, Institute of Complex Systems 7: Biomechanics, Forschungszentrum Julich, Julich, NRW, Germany, r.merkel@fz-juelich.de
Gunter Kerst, Dept. of Pediatric Cardiology, Unikilnik RWTH Aaachen, Aachen, Germany, gkerst@ukaachen.de
Jaime Vázquez-Jiménez, Dept. of Pediatric Cardiac Surgery, Unikilnik RWTH Aaachen, Aachen, Germany, jvazquez-jimenez@ukaachen.de
Mark Spehr, Dept. of Chemosensation, Biology II, RWTH Aachen University, Aachen, Germany, M.Spehr@sensorik.rwth-aachen.de
Stefan Jockenhoevel, Dept. of Biohybrid & Medical Textiles RWTH-Aachen, Aachen, Germany, jockenhoevel@ame.rwth-aachen.de
Petra Mela, Dept. of Biohybrid & Medical Textiles RWTH-Aachen, Aachen, Germany, mela@ame.rwth-aachen.de

Atrioventricular (AV)-block is a pathological condtion of the conductive system of the heart that in severe cases results in the absence of ventricular beating. AV-block affects aproximatly 1 in 14.000-20.000 live births and requires the implantation of a electrical pacemaker to ensure the survival of the newborn. Although successful in restoring cardiac function, these pacemakers can suffer from a multitude of complications. This often result in pediatric patients needing several dangerous, invasive reoperations during their early lifetime. The aim of the BioPacer project is to create a permanent, tissue-engineered solution for AV-block, especially in pediatric patients.

Constructs were created by generating fibrin micro-fibers encapsulating cardiomyocyte and endothelial cells. These were paired with fibers containing fibroblasts, and cultivated in contact with each other over the whole length. After two weeks of cultivation the two fibers fused into one ~250 µm diameter fiber. Cardiomyocytes on the inside of this fusion beated synchroniously over the entire 2 cm length of the fiber.

The morphology of the constructs was further evaluated by immunohistochemical stainings for connexion 43, sarcomeric alpha actinin and CD31 and two-photon imaging. The analysis revealed i) a network of longitudinally oriented cardiomyocites with connexin 43 positive junctions along the whole fiber's length; ii) endothelial capillary like structures and iii) a collagen layer completely surrounding the two, now fused, fibers. Further, electrophysiological recordings showed conduction of an electric input signal up to three Hz with a conduction velocity of 24.49 ± 0.78 cm/s. This velocity would result in a physiological signal delay of 122 ms over the forseen construct length of 3 cm to bypass the AV-node. Overall these results indicate that the BioPacer meets all the functional requirements in vitro, future research will focus on the in vivo evaluation of the construct.

Impact of Different Cell Types and Sources in the Development of Vascular Structures

Caroline Kniebs, Department of Biohybrid and Medical Textiles (BioTex), AME-Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany, kniebs@ame.rwth-aachen.de

Franziska Kreimendahl, Department of Biohybrid and Medical Textiles (BioTex), AME-Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany, kreimendahl@ame.rwth-aachen.de

Marius Köpf, Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany, mkoepf@ukaachen.de

Horst Fischer, Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany, hfischer@ukaachen.de

Stefan Jockenhoevel, Department of Biohybrid and Medical Textiles (BioTex), AME-Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany, jockenhoevel@ame.rwth-aachen.de

Anja Lena Thiebes, Department of Biohybrid and Medical Textiles (BioTex), AME-Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany, thiebes@ame.rwth-aachen.de

Tissue Engineering aims at providing an alternative to donor organs through *in vitro* production of bioartificial tissues or organs. This study deals with the development of a bioartifical tracheal graft using 3D bioprinting techniques. We developed a pre-vascularized transplant by endothelial cells and supporting cells to ensure adequate nutrient and oxygen supply after transplantation.

The focus of this study is the quantification of angiogenesis and thus the formation of vascular structures while comparing different supporting cells from different tissue sources. To our knowledge, there are no structured studies comparing the behaviour of different supporting cells in (printable) hydrogels. A printable hydrogel consisting of 0.5% agarose and 0.5% collagen (0.5AGR0.5COLL) was used in addition fibrin gel as a control. Human dermal fibroblasts (HDFs), human nasal fibroblasts (HNFs), human mesenchymal stem cells from umbilical cord (WJ MSCs), adipose tissue (AD MSCs), and femoral bone marrow cells (BM MSCs) were established as angiogenesis-supporting cells in this study. They were isolated and cultured with endothelial cells from the human umbilical cord (HUVECs) in a concentration of $1x10^6$ cells/cell type/350 µl hydrogel as co-cultures for 14 days at 37 °C, 5 % CO_2 under water vapour saturated atmosphere. The developed vascular structures were detected by CD31 antibody staining with a two-photon laser scanning microscope. From these 3D-images, the parameters volume, area, length and number of branches of the structures were statistically evaluated.

In both, fibrin gel and 0.5AGR0.5COLL hydrogel gel, tubular, branched networks of vascular structures were formed in all tested co-cultures. In general, we observed higher values for volume, area, length and the number of branching points of the vascular structures in fibrin gels. Still, also in 0.5AGR0.5COLL formation of capillary-like structures was proven. This study describes the first step to a printable, pre-vascularized scaffold of endothelial and supporting cells for tracheal substitutes.