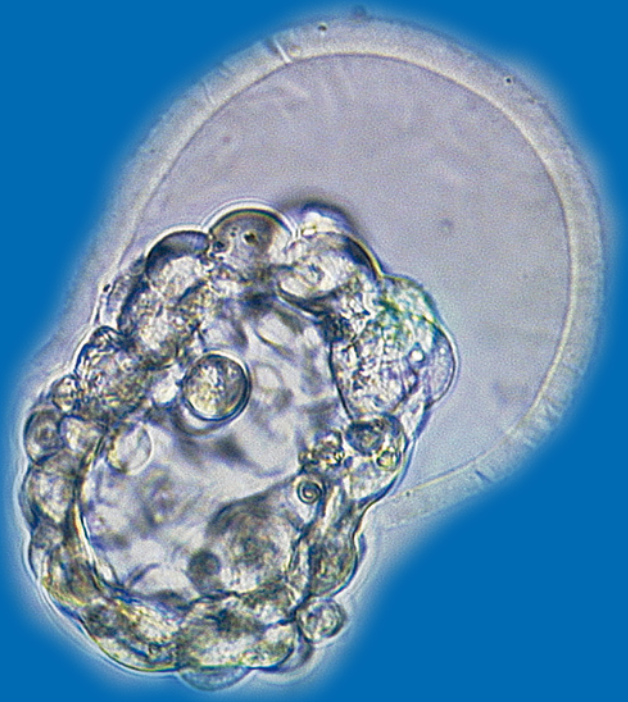
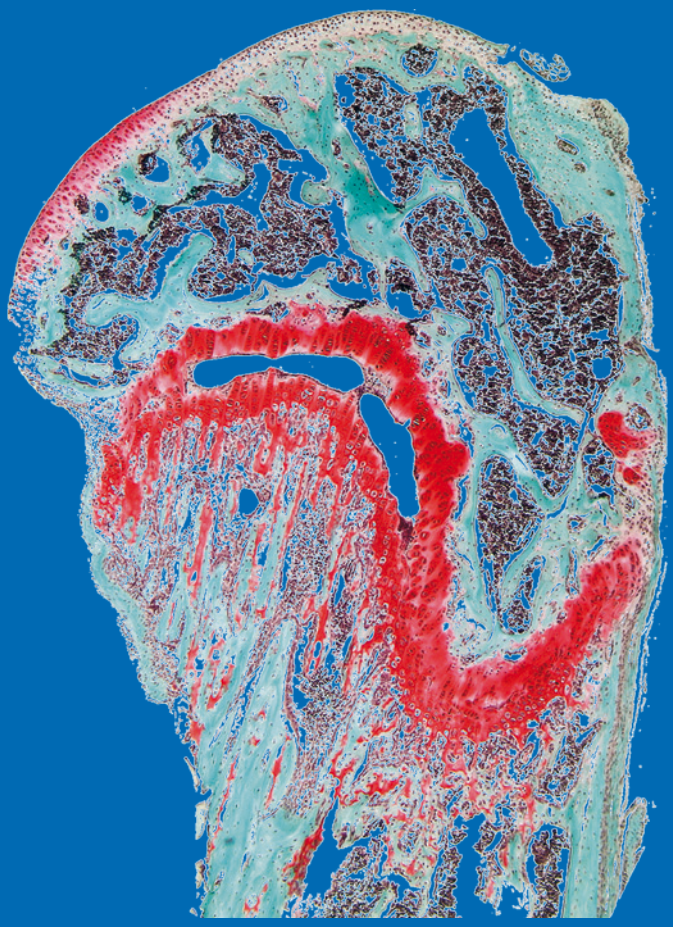
Biointerface Laboratory at Helmholtz-Institute for Biomedical Engineering

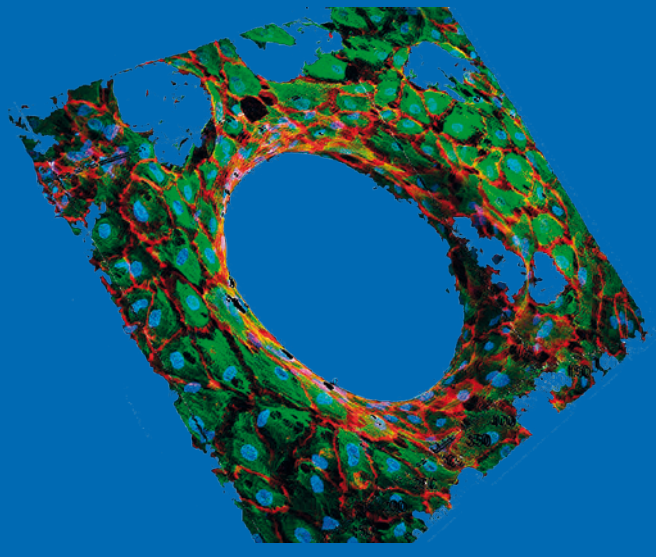
RWTH AACHEN UNIVERSITY

Faculty of Medicine

Cell-Material Interactions: Translating Basic Science Into Clinical Applications







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Cover Figures: *Top, hatching blastocyst penetrating the zona pellucida; Center, breaking bone - epiphyseal lysis in a genetic mouse model deficient in fetuin-A protein; Bottom, making bone – co-culture of bone stromal cells with endothelial cells on a porous scaffold creates highly organized bone-like tissue.*

Introduction

These past year collaborative projects with partners were finally published. We contributed to the discovery by an international team of researchers that the scavenger receptor MARCO, which is expressed in several subsets of naive tissue-resident macrophages mediates in sensing adenoviral infections thus triggering an immune response [4]. Thus, MARCO may influence antiviral innate responses in a virus type-specific manner, which may determine the delivery route of adenoviral gene delivery in live animals and humans.

Together with engineers from RWTH Aachen we applied electrical impedance spectroscopy of single cells in hydrodynamic traps, a versatile method, which is used to study cell integrity, but never before in single mouse oocytes [5]. Ultimately this method may be employed to judge the quality of oocytes destined for *in vitro* fertilization.

Laura Brylka published the results of her PhD work, which she performed in close collaboration with researchers from Hamburg and Cologne [13].

Sabine Neuss-Stein has published in »Angewandte« collaborative work with Andrij Pich's lab on nanogel coatings for taylored biointerfaces [7].

The EU International Network for Training on Risks of Vascular Intimal Calcification and roads to Regression of Cardiovascular Disease (INTRICARE) had its kickoff in March 2017 and most early stage researchers, i.e. PhD students funded by this project have started their work. We also secured a grant within a new Transregional Collaborative Research Center of the German Research Foundation (DFG) addressing reno-cardiovascular interactions underlying enhanced cardiovascular risks in patients with chronic kidney disease (CKD). Our work will focus on circulating high molecular weight protein-mineral complexes triggering soft tissue calcification.

Laura Brylka has left the group after a 7 year stint, first as student aid, next as master student, then as a PhD student, to join as a post-Doc the Hamburg lab of Thorsten Schinke. We wish her well and will stay in touch!

Sarah Peglow returned to Fachhochschule Bonn-Siegen to complete her MSc course work.

Stephan Reinhold completed his MSc and started PhD work as an early stage researcher of the INTRICARE consortium at University of Maastricht.

Electrical Impedance Spectroscopy as Indicator for Oocyte Quality

MSc Carlo Schmitz
Dr. Julia Floehr

Oocyte *in vitro* fertilization (IVF) is an established procedure in human and animal assisted reproduction techniques (ART). IVF critically depends on the quality of oocytes, especially on the state of the *zona pellucida* (ZP), a gelatinous outer layer of extracellular matrix that spontaneously hardens and becomes impenetrable to sperm during oocyte isolation and culture. Oocyte quality is typically judged microscopically by an experienced observer. Optical inspection methods are however observer-biased and thus subjective, time consuming, and they require specialized equipment. Thus, observer-independent characterization methods are highly desirable.

With respect to miniaturization, integration and portability, increased focus has been devoted to electrochemical biosensors with particular interest to electrical impedance spectroscopy (EIS). In cooperation with the group of Prof. Uwe Schnakenberg a novel microfluidic chip for trapping and EIS characterization of single mouse oocytes was developed [5]. Figure 1 shows the top-view of the chip, which mainly consists of a microfluidic channel with four embedded trapping sites defining the detection regions of the device. The trap structures are arranged along the main inlet microchannel and featuring a narrow cross connection region to the outlet channel (hydrodynamic trap). Four microelectrodes (12 μm * 22 μm) are located inside one trap. The four black bars in the pictures correspond to the interconnection lines for the four electrodes. Two electrodes for impedance measurement are pinpointed on the bottom and two on the side walls of the trap, respectively.

In our study, we analyzed the influence of the ZP on the electrical impedance response. Zona-intact oocytes had an overall smaller increase in impedance compared to the zona-free oocytes with respect to empty traps, as depicted in figure 2. The smaller impedance change is induced by the high conductivity of the ZP. The frequency of highest sensitivity was determined to be around 100 - 200 kHz. The results show that EIS is highly sensitive to the ZP structural

changes, suggesting impedance as a novel, non-destructive criterion to judge the quality of oocytes meant for IVF. For further experiments, the device will be improved by an optimized layout.

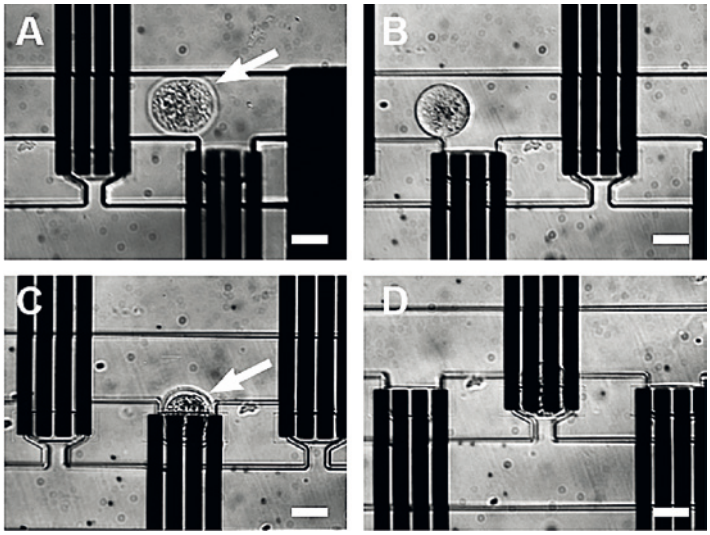

Fig. 1: Single mouse oocytes trapping in the microfluidic device. *(A) Oocytes with surrounding ZP within the microchannel before trapping. (B) Zona-free oocyte in the microchannel before trapping. (C) Successfully trapped zona-intact oocyte. (D) Trapped zona-free oocyte, according [5]. Scale bars are 50 µm.*

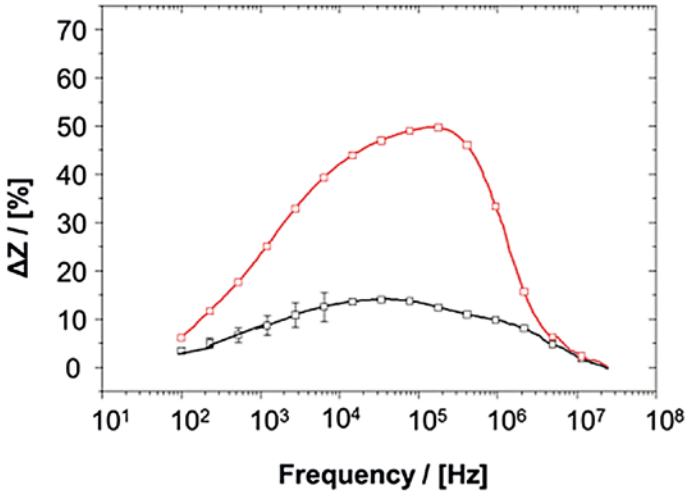

Fig. 2: Normalized impedance change *of ZP-free (red top curve) and ZP-intact (black bottom curve) mouse oocytes with respect to empty traps, according [5].*

Stem Cells and Tissue Engineering

Prof. Dr. Sabine Neuß-Stein

In the working group "Stem Cells and Tissue Engineering" the main issues being addressed are (i) the choice of promising cell types and (ii) the choice of suitable biomaterials for specific tissue engineering applications, as well as (iii) vascularization of three-dimensional biomaterial scaffolds and (iv) mineralization efficiency for bone tissue engineering.

In the past, mesenchymal stem cells (MSC) were used from different species (human, murine, ovine) and origins (bone marrow, umbilical cords, adipose tissue) and in 2017 we fully characterized MSC from an additional and ontogenetically early origin, the chorionic villi of placenta [16]. Compared to MSC from other sources, chorionic villi-derived MSC possess an extended life span due to a delay in replicative senescence and aging as a result of improved telomere length maintenance. This makes chorionic villi-derived MSC an attractive cell type for Regenerative Medicine.

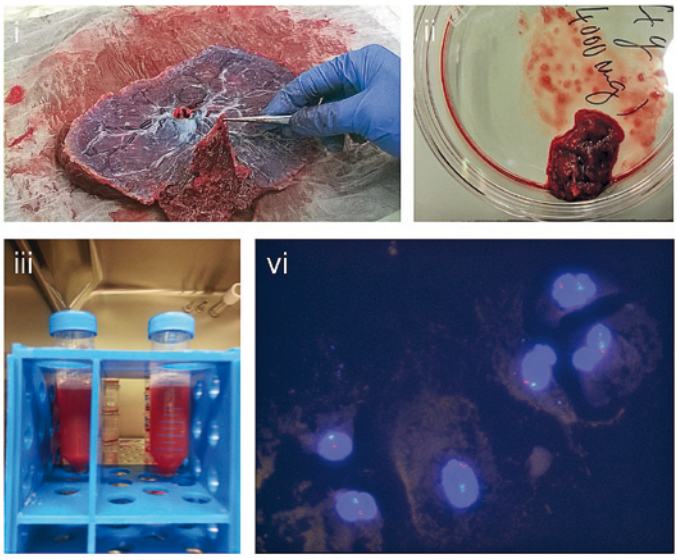

Fig. 3: Isolation of human chorionic villi-derived mesenchymal stem cells (CV-MSC) from human placenta. *i) Removal of the amnion to access the chorionic plate, ii) pieces of chorionic villi dissected from human placenta, iii) collagenase digestion of chorionic villi; iv) interphase FISH analysis using a X/Y dual color probe to identify male cells in male/female mixed placenta tissue. X chromosome fluorescence is green while Y chromosome fluorescence is orange. Scale = 50 µm [16].*

Besides comprehensive analyses on cell characteristics [16] the group optimized a biomaterial-based stem cell recruitment system with hepatocyte growth factor (HGF) being released from silk membranes by cleavage of a tPA restriction side via serine proteases in wounded areas. After a successful proof-of-concept where endogenous MSC are guided to wounds in a mouse model [1], the group is currently adopting the protocols for the human system and could already produce (in mammalian cells) and purify the secreted HGF with first successful pilot experiments on its functionality.

In addition, the group gained more experience in novel biomaterials, e.g. in polymers for drug delivery systems [14], polymers for implant coatings allowing for both, cell adherence and microbe repellence [7] and in high strength ceramics and polymers for bone tissue engineering [9, 15, 17]. For larger three-dimensional, tissue engineered bone constructs, co-cultures are needed, providing bone building cells (e.g. MSC) and endothelial cells (e.g. human umbilical vein endothelial cells, HUVEC) for capillary-formation for future warranty of nutrient supply. Both cell types have to be in proper cross-talk and we could already optimize culture conditions in a way that MSC show increased mineralization, while HUVEC form capillary-like structures.

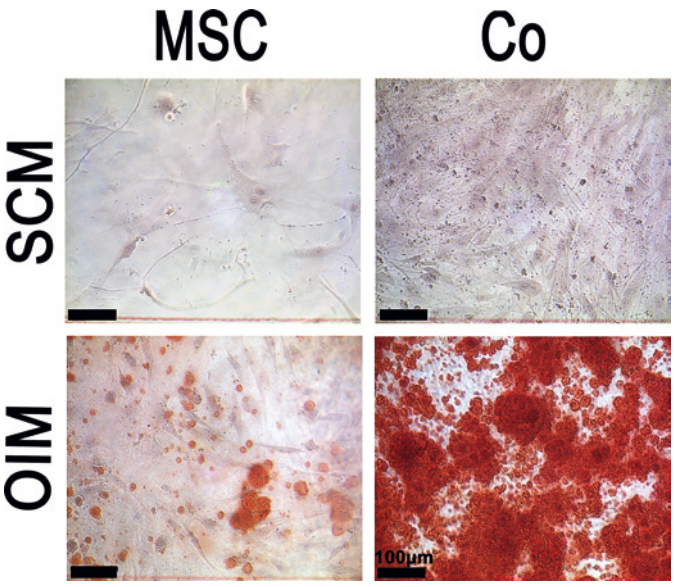

Fig. 4: Enhanced osteogenic differentiation of MSC after co-culture with endothelial cells. *Human MSC were cultured in mono- (MSC) or co-culture (Co) either in stem cell expansion medium (SCM) or osteogenic induction medium (OIM). Success of differentiation/presence of calcium accumulations was visualized via Alizarin red stainings. A co-culture with endothelial cells enhanced osteogenic differentiation of MSC compared to monoculture conditions. Scale = 100 µm.*

Growth Factor-functionalized Silk Membranes Support Wound Healing In Vitro

MSc Michaela Bienert

Chronic wounds represent a serious problem in daily medical routine requiring improved wound care. Silk of the domesticated silkworm *(Bombyx mori)* has been used to form a variety of biomaterials for medical applications. *B. mori* was genetically engineered to produce silk functionalized with growth factors. In this study EGF-, FGF-, KGF-, PDGF- or VEGF functionalized silk membranes were compared to native *B. mori* silk membranes without growth factors for their ability to support wound healing *in vitro*.

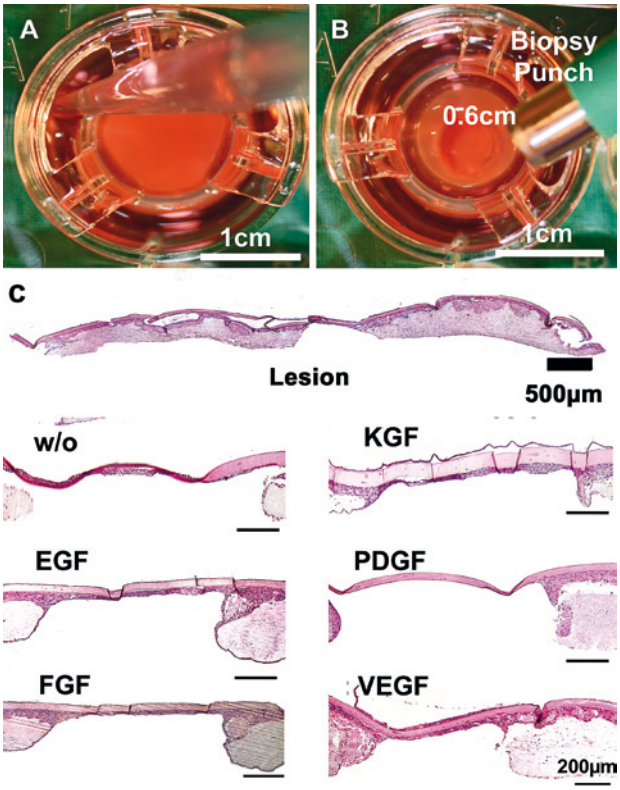

Fig. 5: Growth factor-functionalized silk membranes in dermal equivalents (DE) in vitro. *(A) Dermal fibroblasts in collagen gels covered by keratinocytes are exposed to the air by removing the medium from the top compartment of the transwell system. (B) Biopsy-punch lesion with a diameter of 0.6 cm and an area of 1.13 cm2. Lesions were covered with silk membranes functionalized with FGF, EGF, KGF, PDGF, VEGF or without growth factors. (C) 17 days after setting the lesion, gels were embedded in paraffin, sliced longitudinally and stained with HE. Keratinocytes (dark pink) grow underneath the silk layer/on top of the collagen embedded fibroblasts (light blue); scale bar 500 µm. HE staining of silk covered DE with growth factors EGF, FGF, KGF, PDGF, VEGF or without growth factors; scale bar 200 µm.*

Our *in vitro* wound healing studies showed as a trend that EGF-, FGF- and VEGF-functionalized silk enhanced wound closure compared to silk without growth factors.

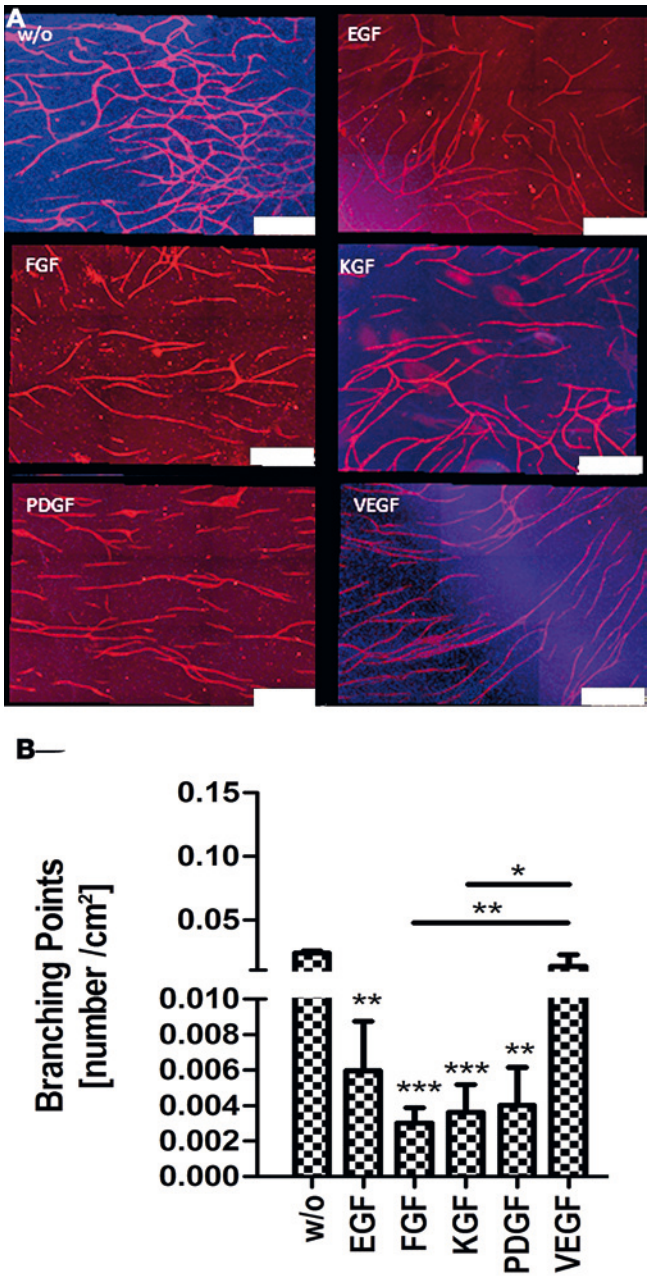

Fig.6: Angiogenic capacity of growth factor-functionalized silk membranes. *(A) Capillary-like structure formation on silk membranes without growth factors or functionalized with FGF, EGF, KGF, PDGF, VEGF. or silk without growth factors. HUVEC stained with anti-CD31 (red) and counterstained with DAPI (blue). Scale bar indicates 500 µm. (B) Quantification of branching. All values are normalized to relative cell numbers, error bars represent the standard deviation n=3, t-test, p=0.05.*

In angiogenesis studies, VEGF-functionalized silk membranes and native silk membranes significantly outperformed the other growth factor-functionalized silk membranes by producing longer and more branched capillary-like structures.

Murine macrophages on all silk membranes secreted cytokines and chemokines promoting neutrophil infiltration (CXCL1) and macrophage infiltration (MCP-1) whereas the proinflammatory cytokines IFN-γ, IL-6 or TNF-α could not be detected (figure 7).

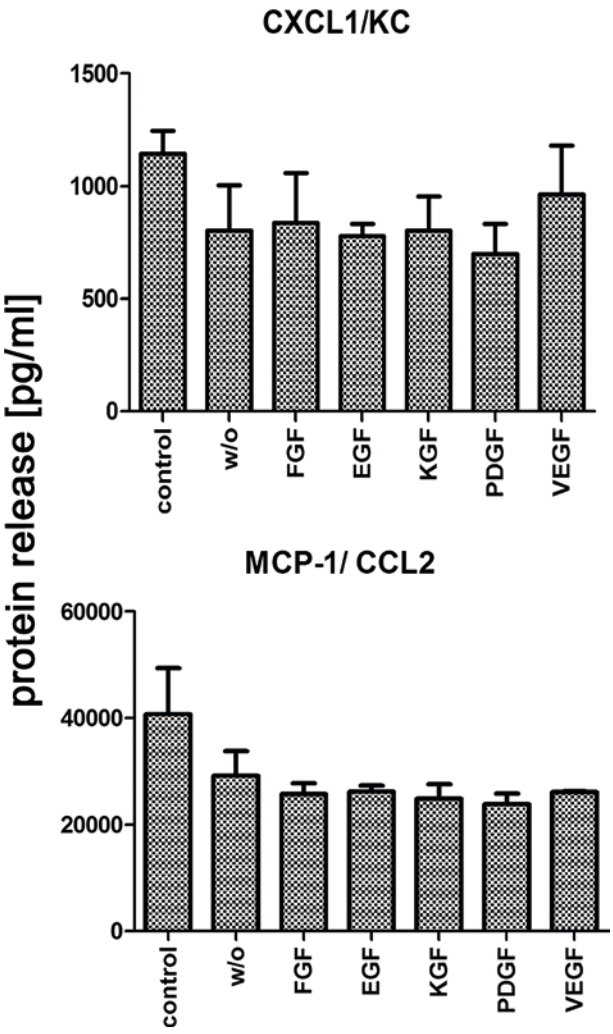

Fig. 7: Macrophage cytokine and chemokine secretion on different silk membranes. *Silk func-tionalized with growth factors FGF, EGF, KGF, PDGF, VEGF and silk without growth factor were incubated with macrophages for 1 day. Macrophages on cell culture plastic served as a control. CXCL1/KC, MCP-1/CCL2 chemokines were quanti-fied by flow cytometry using FlowCytomix®. Error bars represent the standard deviation n =3, t-test, p = 0.05.*

Thus, we here introduce *Bombix mori* derived, growth factor-functionalized silk membranes as promising biomaterials for wound healing therapies.

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Team in 2017