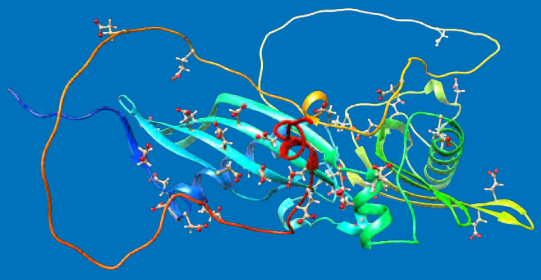
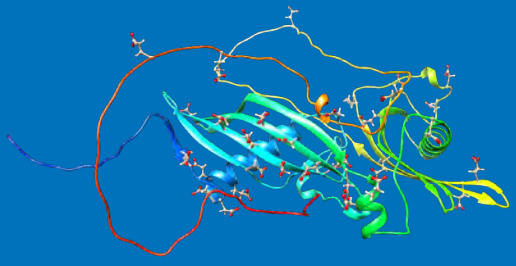
Biointerface Laboratory at Helmholtz-Institute for Biomedical Engineering

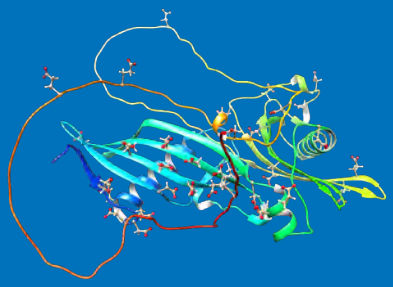
RWTH AACHEN UNIVERSITY

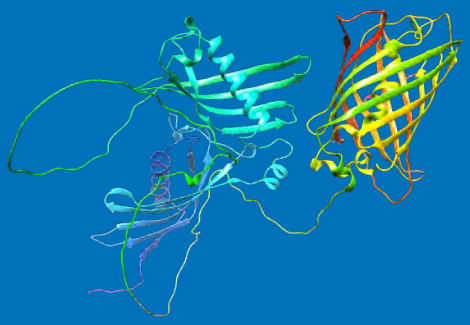
Faculty of Medicine

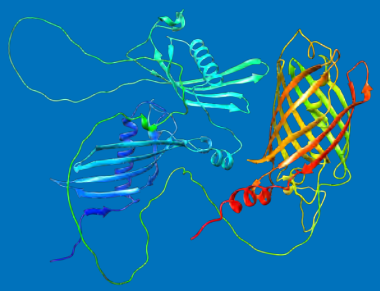
Cell-Material Interactions: Translating Basic Science Into Clinical Applications











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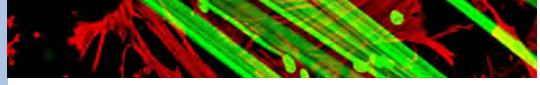


Cover Figures: Recombinant Fetuin-A Fusion Proteins modelled with Alphafold

Introduction

Willi Jahnen-Dechent, Professor

Like the year 2020, 2021 was tainted by COVID. Just when we thought the pandemic was over after two long years, and the outlook was bright, we found ourselves in a political nightmare with war striking in the midst of Europe. Against dramatic worldwide challenges our little world of science may seem insignificant, but it isn't. Brilliant research has ended the COVID threat by providing vaccines in "lightspeed", and research in other areas may help alleviate the environmental and societal ills that drive conflicts around the world. Can fetuin or biomaterial research make a difference? I don't know, but I do know that our research like any other is about reasoning and embracing the unknown – something that frightens all demanding "simple answers". There are none! So once again let us share progress made in the past year, however little it may be.

We secured continued external funding to study the structure and function of the mineral chaperone fetuin-A, the development of a miniaturized device for calcification testing, the role of periodontal ligament cells in alveolar bone healing, bio-functionalized microgels and their influence on mesenchymal stromal cell differentiation, ceramic-based implants for cardiovascular tissue engineering, all of which is presented below.

After many successful years of research on the role of fetuin-B in reproductive biology, Julia Floehr, Carlo Schmitz and Seynab Sadr all expertly finished their projects. They have already left for new jobs or will soon take employment elsewhere. Fetuin-A researchers Sina Koeppert left after completing her PhD defense "with distinction". Andrea Gorgels completed all PhD exams just in time before taking maternal leave. We thank all these great colleagues for their contributions in and outside the lab. It is always a bit sad to see established young scientists leave the group. But isn't that what University training is all about? All the more rewarding to see alumni thrive in their new professional and personal careers. We are proud and most importantly, we stay in touch!

Stem Cells and Tissue Engineering

Sabine Neuß-Stein, Professor

Our work continues to focus on three main research topics: (i) mesenchymal stem cells (MSC) and periodontal ligament stem cells (PDL cells) in wound healing and tissue regeneration, (ii) bone tissue engineering and (iii) cardiovascular tissue engineering.

Together with the group of Michael Wolf (Clinic for Orthodontics) we identified striking differences in kinase-dependent signaling of periodontal ligament (PDL) cells from the upper and lower jaw to support healing of the alveolar bones. We started a project on understanding the PDL cell-cementum interphase to provide a basis for periodontal-cementum research. Chloé Radermacher presents the results of her Master thesis below. She continues her work as a PhD candidate.

The second phase of our externally funded project with Andrij Pich (Institute of Textile and Macromolecular Chemistry) studies fibrin-based hydrogels containing dextran or extracellular matrix molecules to form functional microgels (figure 1). These microgels are further decorated with growth factors and cytokines to regulate (stem) cell recruitment and differentiation. In addition, lipid-membrane coated microgels housing sensors and DNA are compared against established cell lines to replace lipid-membrane based "artificial cells" for live cells in cell-based assays.

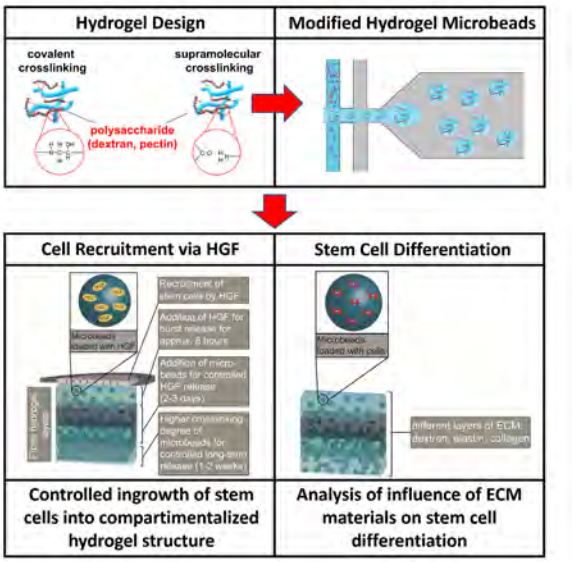


Fig. 1: Development of hierarchical and compartmentalized hydrogels for stem cell recruitment, targeted cell growth and differentiation using microgels.

We also continue our work with our long-lasting cooperation partner Karolina Schickle (Institute of Mineral Engineering) to develop ceramic-based implants for cardiovascular tissue engineering. Here we found that monocrystalline ceramics of different anatomical layers are hemocompatible and have different impact on cell behaviour, in particular on thrombocyte adhesion and activation.

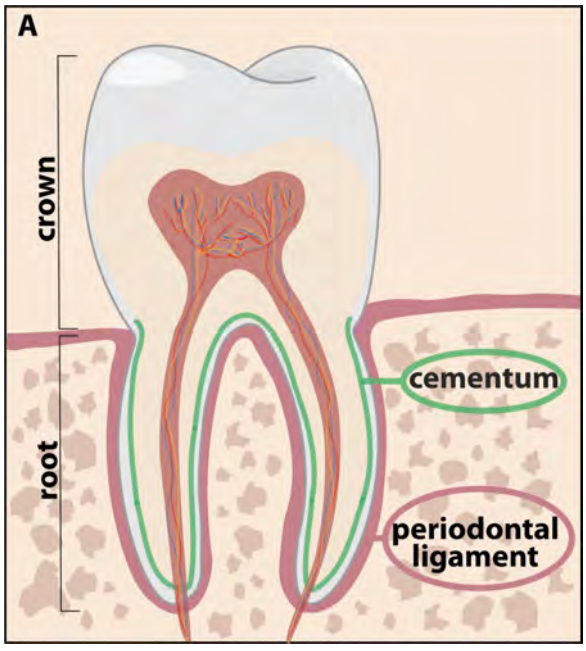
Besides, we develop GMP protocols for translational research within the newly founded FiT-center ("first in translation") jointly operated by the Leibniz Institute for Interactive Materials and the Center for Biohybrid Medical Systems (CBMS), which are located in close proximity on the RWTH Aachen Research Campus "Melaten". FiT addresses the gap between research projects and clinical translation. Specifically, our satellite project studies ceramic surfaces loaded with bioactive molecules to better integrate into bone tissue.

What started out as a fun project fuelled by a lot of enthusiasm and some crowd funding has quickly turned into an exciting biomaterial project for bone tissue engineering. Learn more about Anna Bartz's work with spider silk below and watch her on YouTube (https://www.youtube.com/watch?v=1YLWi2hcqkU).

Understanding the PDL cell/ cementum interphase, their interplay under mechanical stress and periodontal remodelling to provide a basis for periodontal-cementum research.

Chloé Radermacher, PhD candidate

Orthodontic treatments together with their resulting side effects play an important role in the 21st century because of psychosocial, functional, and dental issues. These treatments cause a pathological process for up to 46% of patients depending on the brace type. In this case, the patient is affected by inflammatory root resorption, which can lead to permanent loss of dental root structure. Today, there is no mechanistic explanation for orthodontically inflammatory root resorption, but the following factors are implicated: mechanical forces, the morphology of tooth roots, alveolar bone, periodontal ligament, cementum, and several biological messengers. The cementum is a key tissue in the initial periodontal development process as well as in regeneration after periodontal diseases. Indeed, this mineralized tissue is covered by root lining cells called cementoblasts. These cells can be stimulated by mechanical or biological signals to build up new cementum and repair tooth roots. The project aims at investigating the role of cementoblasts and their interaction with the periodontal ligament. My master thesis´ goal is to establish an isolation method of cementoblasts and periodontal ligament cells from the same patient and the characterization of these cell types.



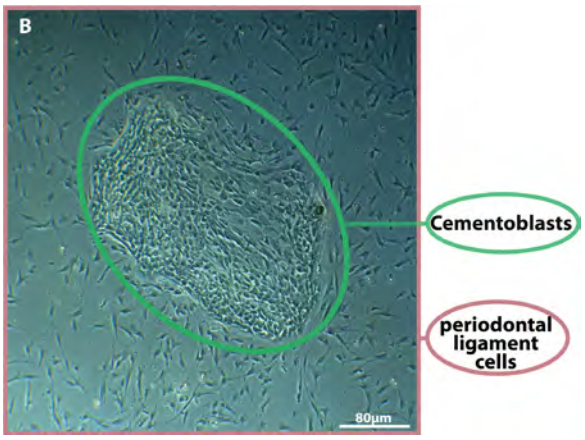


Fig. 2: (A) Anatomical scheme of the tooth with particular focus on the tissues of interest: cementum and periodontal ligament. (B) Cementoblast colony surrounded by periodontal ligament cells three days after isolation. Scale bar: 80 μm

New concepts in regenerative medicine - spider silk-based scaffolds for bone replacement strategies

Anna Bartz, PhD candidate

Spider silk has special biomedical properties (very high tensile strength, high elasticity, antibacterial effect, cyto- and biocompatibility, degradability, heat stability) that make it particularly interesting as a matrix for supporting cell functions in bone replacement material. The aim of our research project is to produce a functional biohybrid, three-dimensional construct, consisting of a carrier material (silk from spider and/or caterpillar) surrounded by a hydrogel (fibrin- or collagen-based) with integrated bone cells, which are differentiated in vitro from mesenchymal stem cells (MSC) or periodontal ligament cells (PDL cells), as well as capillaries from co-cultured endothelial cells for subsequent connection to the vascular system.

As there are no pre-fabricated devices available on the market which could be used for "milking" the spiders and for winding the silk threads onto miniature frames, the project had to start from scratch by developing and testing several appliances which are currently optimised with support from the company Status pro Maschinentechnik GmbH. This will allow us to wind the spider silk in an exact and reproducible way onto the weaving frames.

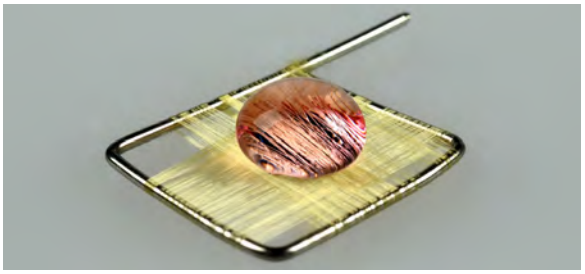


Fig. 3: Miniature weaving frame with the machine-wound spider silk used in this study (Photo: University Hospital RWTH Aachen)

First results demonstrate that spider silk is cytocompatible and that the MSC remain long-term viable when adhering to the spider silk fibres. Promising results were also achieved with MSC embedded in a fibrin-based hydrogel surrounding the silk fibres.

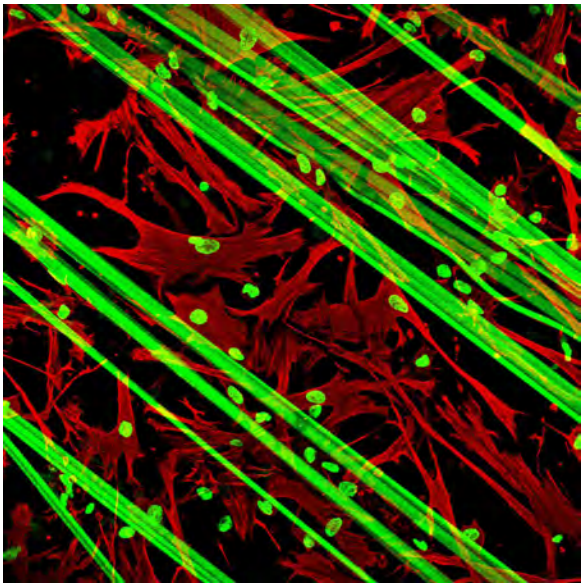
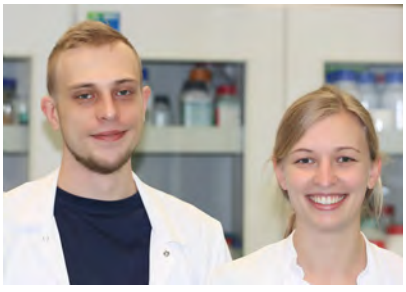


Fig. 4: Two-Photon-Microscopy (2PM) of spider silk in combination with MSC embedded in a fibrin-based hydrogel

Another novel scaffold material for culturing bone replacement biohybrid scaffolds,is tested in parallel: we currently use fish skin xenografts from Atlantic cod to verify its suitability for osteogenic differentiation of MSC. First results indicate that the fish skin has very good cytocompatibility and that the MSC preferentially colonize the fish scale side and migrate into the fish skin. Also in this case, the combination with a fibrin-based hydrogel supported a more effective cell growth and proliferation. First results indicate that spider silk and fish skin are promising biomaterials for bone replacing strategies.
In this research project, we collaborate with different cooperation partners: Aquazoo Löbbecke Museum Düsseldorf, Tierpark + Fossilium Bochum, Clinic for Orthodontics at RWTH Aachen University, Institute for Textile Machinery and Textile High Performance Materials Technology (ITM) at the University of Dresden, Status Pro Maschinenmesstechnik GmbH and the Icelandic Startup Kerecis.

Structure-function analysis of recombinant Fetuin-A



Christian Hasberg, Camilla Winkler, PhD candidates

The word protein was derived from the Greek word “proteios” for basic, primary. The all-important structure of a protein is determined by the sequence of its building blocks, the amino acids. After synthesis in the cells, proteins fold from a thread of amino acids into helices and loops, and ultimately bend into defined three-dimensional structures, which determine the function of a protein.
Two proteins of the cystatin superfamily, fetuin-A and fetuin-B are the focus of our structure-function research. Both fetuins are similar in structure and consist of three domains. The first two domains are cystatin-like CYS domains, which consist of an alpha helix surrounded by 5 anti-parallel beta sheets. The third domain is called the C-terminal region CTR. Fetuins are predominantly made in the liver and secreted into the blood stream. Despite their structural similarity, the functions of fetuin-A and fetuin-B strongly differ. Fetuin-A is a binding protein regulating mineralization and scavenging excess calcium phosphate from the circulation and thus protecting against ectopic calcification. Fetuin-B is a potent inhibitor of the oocytes-specific proteinase ovastacin, which regulates oocyte fertility.

For structure-function studies our group produced with the help of recombinant protein technology fetuins and related proteins in E. coli bacteria, in chinese hamster ovary (CHO), and in human embryonic kidney (HEK) cells. The proteins possess binding tags to facilitate protein purification and fluorescent tags to aid their detection.

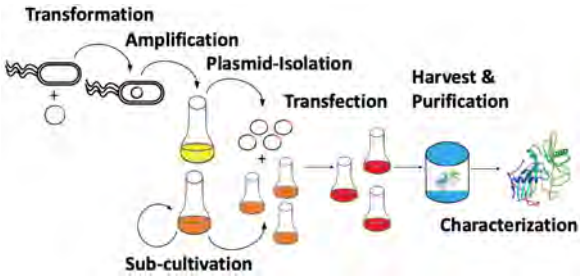
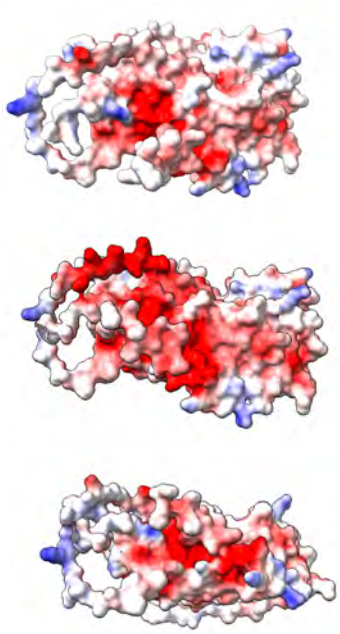


Fig. 5: Scheme of recombinant protein expression and purification.

Using established molecular biology routines, we substitute amino acids deemed important for protein function. For instance, we replaced cysteine residues linking fetuin CYS1 to CTR to study the influence of this domain-bridging disulfide bond on overall protein stability. Other protein mutants study the role of phosphorylation and glycosylation in fetuin function. Up to now, we produced more than 50 fetuin variants, which allow us to pinpoint features regulating the function and activity of these proteins.

Although the amino acid sequence for the fetuins has been known for a long time, no complete 3D-structure of fetuin-A was known. Recently,we published the structure of fetuin-B in collaboration with Proffs Gomis-Rüth and Stoecker from Barcelona and Mainz, respectively. Based on the fetuin-B structure we could also model a complete 3D structure of fetuin-A. In mid-2021 the AlphaFold structural modeling suite revolutionized the visualization of almost any protein purely based on the amino acid sequence. For the first time we can now any visualize fetuin variants to make informed decisions about optimized versions of functional fetuins.
Fig. 6: Computer models of recombinant fetuin-A variants. Small amino acid sequence changes in these proteins dramatically change their surface charge and mineral binding capacity.



Vessel-On-A-Chip: A miniaturized device for calcification testing

Aaron Morgan, PhD candidate

Biohybrid implants (those made from both biological and non-biological components) are a promising development in medical technology but often suffer from issues like thrombogenesis (the formation of blood clots) and calcification (calcium build-up, causing tissue hardening). Testing for these types of materials can be difficult and expensive, due to the nature of the materials, fabrication methods, or special reagents. To facilitate the testing and validation of these materials, we have developed a miniaturized calcification chamber that recreates the flow conditions found in arterial blood vessels.

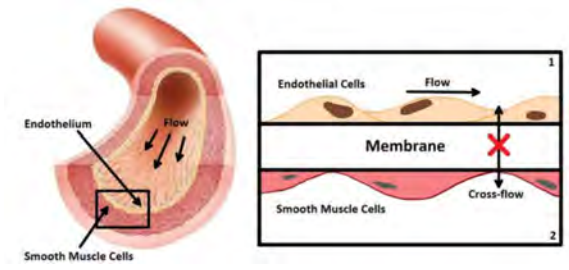


Fig. 7: (Left) Blood vessel cross-section with the cells of interest indicated. (Right) Simplified schematic view of the cell environment recreated in the chip.

This chamber holds a material sample between two fluid channels. Each fluid channel may contain a different medium and be pumped independently, though the device is designed to be driven by a single pump. The "high flow" side uses flowing medium to exert physiological shear stress onto the material surface (1-12 dyne/cm2), while the "low flow" side circulates the medium without exposing the material to significant shear stress. This more closely reproduces the physiological conditions of both the blood-facing layer, the endothelium, and the smooth muscle cells underneath.

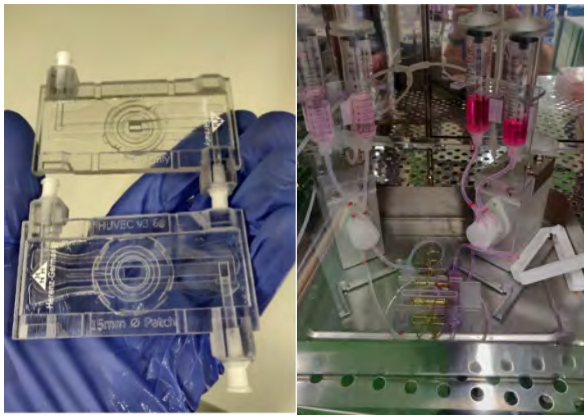


Fig.8: (Left) One complete chip, ready for use. (Right) The medium in the chips is driven using two syringe pumps, each with unique cell medium.

Using this device, we conducted calcification experiments to compare the device with a larger, more expensive testing device that is routinely used. The results showed comparable positive calcification in samples of bovine pericardium, while showing no signs of calcification in samples on PCU (negative control). Additionally, the bovine pericardium was then sectioned to determine the location of the calcification. Using von Kossa staining, we can see that the calcified lesions are located just below the outermost layer and can clearly be seen in the deeper layers of the material.
Going forward, we plan to work alongside other groups to test a wide range of biohybrid materials and hydrogels. We intend to investigate the mechanisms that allow calcification to begin using live-imaging and fluorescent markers, specifically through insights into the physiological and pathophysiological endothelium and the role this barrier plays in the process.

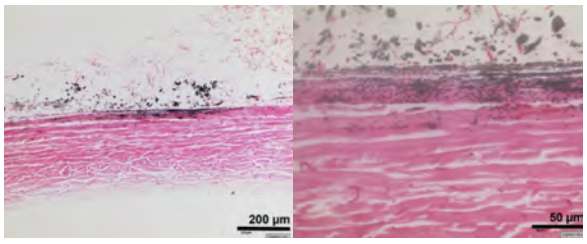


Fig. 9: Bovine pericardium (heart tissue) sections after seven days in the chip, under flow. Calcified lesions can be seen on the side exposed to calcification medium, while the other side remains uncalcified.

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Team in December 2021 at the "Three-Country-Corner" (D-B-NL) in Aachen Town Forrest