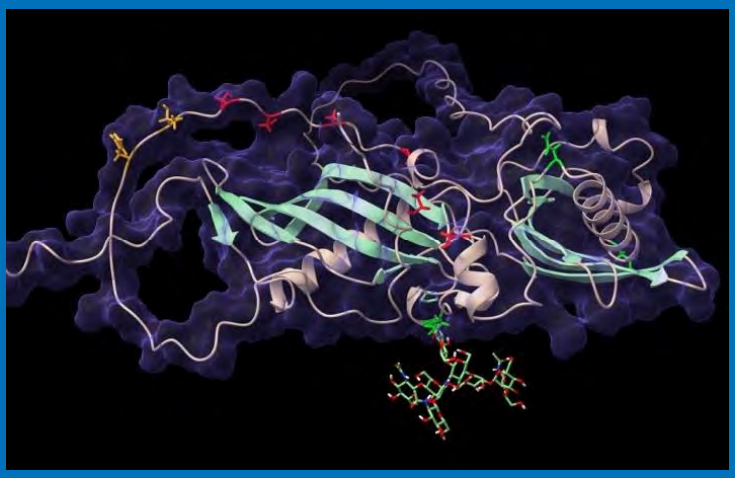
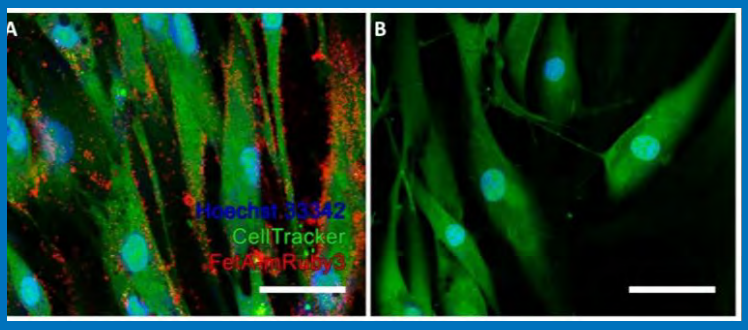
Biointerface Laboratory at Helmholtz-Institute for Biomedical Engineering

RWTH AACHEN UNIVERSITY

Faculty of Medicine

Cell-Material Interactions: Translating Basic Science Into Clinical Applications





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Cover Figures:

***Cover Figure Top**, it happens to the best, it happens to the rest – and it takes a while to recover from COVID, in this case 10 days to be exact, until the antigen test turned negative. Back to work!*
***Second from above**, getting an ever more detailed view of our poster child protein - Fetuin-A modelled with Alphafold including post-translational modifications glycosylation and phosphorylation*
***Third from above**, back to work as well for recombinant Fetuin-A-mRuby fusion protein, an excellent probe to stain early stage mineralization in cell based mineralization and calcification assays.*
***Bottom**, woman without fear - PhD student Anna Bartz casually handling a female Nephila spider. This animal will procure hundreds of meters of silk thread for three-dimensional scaffold materials if handled tender and with care.*

Introduction

Willi Jahnen-Dechent, Professor

We finally made it through the COVID pandemic, taking shots and required precautionary measures. Still, many of us eventually succumbed to the virus and whoever has not, will certainly do in the near future. COVID has lost its sting. I wish we could say the same of the war in Ukraine, which hopefully will also end sooner than later. In this case however, precaution and protective measures don't cut it – we just have to be patient, resolved and united until this madness ends.
Right here and now we continue to study the structure and function of the mineral chaperone Fetuin-A, the development of devices and assays for calcification testing, the role of stem cells and precursors in tissue healing as well as the study of innovative biomaterials to improve tissue engineering, all of which is presented below.
We welcome Sarah Kellner to the office. She took over from Renate Sous who for 20 years flawlessly administrated in our group personnel, reporting, student teaching, exams, research funds and social events. Renate will fill in once a week to secure a smooth transition. A big Thank You, Renate! Sabine Neuß-Stein and her team moved from the Institute of Pathology to the Biointerface labs and offices, a very welcome move indeed. This marks a renewed interest in cell-material interactions fully in line with our very label "Biointerface Laboratory".

Stem Cells and Tissue Engineering

Sabine Neuß-Stein, Professor

In 2022, we could further develop our MSC recruitment system for in situ tissue engineering, which avoids the time- and money-intensive conventional tissue engineering approach with isolated and expanded cells that are seeded on a three-dimensional scaffold to substitute a tissue function. The main research focus of the group is still the role of human mesenchymal stem cells (MSC) in wound healing and tissue engineering. To more accurately release MSC chemoattractants and direct the stem cells towards a damaged location, we now use this recruitment approach for endogenous MSC to several scaffold materials based on ceramics and polymers with adjusted elasticity and degradation durations.

Besides, we gained further knowledge on cell types of the alveolar bone, in particular on periodontal ligament stem cells (PDLSC). Hanna Malyaran a PhD student in a project funded inhouse by the Interdisciplinary Center for Clinical Research IZKF showed marked differences in proliferation and differentiation capacity between PDLSC from the upper and lower jaw of the same patient. PhD student Chloé Radermacher went on to show that PDLSC secrete more vascular endothelial growth factor VEGF than MSC and thus support more efficiently endothelial cells in forming capillaries. In addition, we found out that stroma-derived stem cells including MSC from bone marrow and adipose tissue, and PDL cells from upper and lower jaw differentially respond to mechanical forces. This research added to our molecular understanding of mechanobiology the influence of the mechanical environment on cell behaviour in the body. Now that we better understand PDLSC, we turn to the isolation and characterization of human cementoblasts of alveolar bones, a challenge which as part of a project is funded by the Deutsche Forschungsgemeinschaft DFG.

Together with Karolina Schickle from the GHI of the RWTH Aachen, we investigated monocrystalline ceramic scaffolds and could demonstrate that hemocompatibility and endothelialization are related to the atomic composition of the upper surface of the scaffold and we could successfully publish a patent application (EP21722810.5) with ceramic nanoparticles integrated in cardiovascular stents to improve hemocompatibility and reduce the risk of thrombocyte aggregation and activation.

PhD student Anna Bartz continues her crowd-funding campaign to support the study of spider silk as a biomaterial. Anna employs spider silk to produce three-dimensional scaffolds for bone tissue engineering. In her quest for innovative biomaterials Anna recently also included in her cytocompatibility testing fish skin from an Islandic fishing consortium. She demonstrated that MSC and endothelial cells preferentially adhere to specific side of the skin, discriminating the external side originally covered I scales from the side facing the muscle tissue, respectively. Stay tuned to learn more about this exciting biomaterial next year J

Last but not least, we are pleased that PhD candidate Svenja Wein has rejoined the lab following her parental leave. As part of a different DFG-funded research, she keeps working on fibrin-based scaffold materials and vascularization.

Periodontal ligament stem cells vs. mesenchymal stem cells as stromal support for angiogenesis

Chloé Radermacher, PhD candidate

Periodontitis is the most common reason for tooth loss in adults and affects around 11.2% of the world´s population. The periodontal ligament contains stem cells which are known to have similar characteristics to mesenchymal stromal/stem cells (MSC). The aim of the study is to investigate the angiogenic support of periodontal ligament stem cells (PDL cells) from the upper and lower jaw (u-PDL cells and l-PDL cells, respectively), since there is clinical evidence for differential speed and efficiency in wound healing and regeneration processes and angiogenesis in general plays an important role in regeneration processes of wounds. Bone marrow-derived MSC (BM-MSC) served as controls, since they possess a well-known supporting function for capillary formation of endothelial cells. Here, PDL cells from the upper and lower jaw are co-cultured with human umbilical vein endothelial cells (HUVEC) to compare them with each other and with mesenchymal stromal cells. The increased capillary formation has been demonstrated in PDL cell co-cultures compared to MSC co-cultures by immunofluorescence staining (Fig. 1). This phenomenon can be explained by the higher VEGF (vascular endothelial growth factor) secretion of PDL cells compared to BM-MSC. Indeed, 2ng/mg more VEGF was secreted in the medium supernatant of PDL cells than in BM-MSC. Lastly, slightly increased capillary formation was seen in the co-culture with PDL cells from the maxilla compared to the co-culture with PDL cells from the mandible of the same donors. In the present study, we could demonstrate for the first time, that PDL cells are able to support endothelial cells in the capillary formation process. These pilot experiments showed that u-PDL cells have higher angiogenic potential compared to l-PDL cells. This observation may help to explain the clinical observation, that wounds in the maxilla tend to close faster than in the mandible.

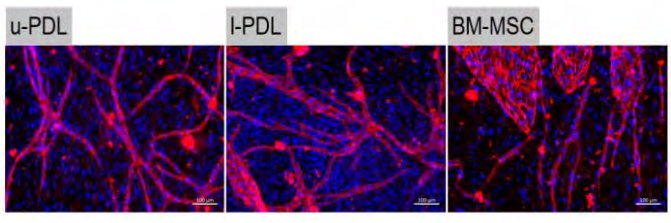

Fig. 1: Representative immunofluorescence pictures from the co-culture of human umbilical vein endothelial cells and periodontal ligament stem cells from the upper (u-PDL) and lower jaw (l-PDL) and from bone marrow-derived mesenchymal stromal cells (BM-MSC). The endothelial cells are stained with CD31 and nuclei of both cell types in co-cultures are stained with DAPI

Comparative analysis of proliferative and multilineage differentiation potential of human periodontal ligament stem cells from maxillary and mandibular molars

Hanna Malyaran, PhD candidate

Clinical experience indicates that wounds in alveolar bone and periodontal tissue heal faster and more efficiently in the maxilla compared to the mandible. Since stem cells are known to have a decisive influence on wound healing and tissue regeneration, the aim of this study was to determine whether differences in proliferation and differentiation of periodontal ligament stem cells from upper (u-PDLSC) and lower jaw (l-PDLSC) contribute to the enhanced wound healing in the maxilla. U-PDLSC and l-PDLSC from the same donor were harvested from the periodontal ligament of extracted human maxillary and mandibular third molars. Cell characteristics of u-PDLSC and l-PDLSC of the same donors were assessed by analysing stem cell markers, proliferation rate and multilineage differentiation and compared to bone marrow-derived mesenchymal stem cells (MSC, Fig. 2). Successful differentiation of PDLSC and MSC towards osteoblasts, adipocytes and chondrocytes was analysed via RT-qPCR and histochemical staining (Alizarin Red, Oil Red O, Toluidine Blue). PDL cells expressed the MSC-markers CD73+, CD90+, and CD105+ and lacked expression of CD34- and CD45-. Proliferation was significantly higher in u-PDLSC than in l-PDLSC, regardless of the culture conditions. Osteogenic (ALP, RunX2 and Osteocalcin) and chondrogenic (SOX9 and ACAN) related gene expression as well as staining intensities were significantly higher in u-PDLSC than in l-PDLSC. No difference in adipogenic differentiation was observed. Thus, we could identify differential cell characteristics of PDL cells from maxilla and mandible of the same donor which might be a first hint towards the mechanism behind the differential wound healing within the upper and lower jaw.

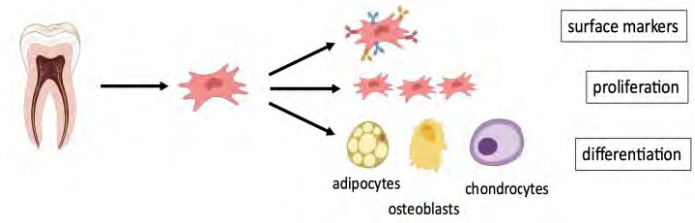

Fig. 2: Periodontal stem cells were isolated from third molars of the upper and lower jaw of the same patient and characterized in terms of surface markers, proliferation and differentiation capacity.

Structure-function analysis of Fetuin-A

Christian Hasberg, Camilla Winkler, PhD candidates

Our research team has a long-standing interest in Fetuin-A protein structure and function. Fetuin-A regulates mineral metabolism especially in conditions of mineral supersaturation. To prevent ectopic calcification, Fetuin-A stabilizes excess calcium phosphate as a colloid and mediates its distribution to target organs or its elimination from the body. Fetuin-A has three folding domains. The first two domains are cystatin-like CYS domains, which include five antiparallel beta sheets covering an alpha helix. The third domain is the C-terminal region CTR, which is intrinsically disordered. Post-transcriptional modifications phosphorylation and glycosylation likely regulate Fetuin-A function (Fig. 3).

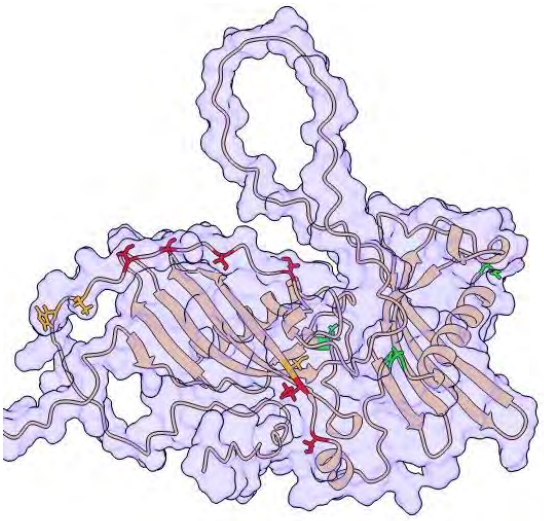

Fig. 3: A model of murine Fetuin-A was developed using the program AlphaFold. A volume model of the peptide backbone is displayed in transparent purple. Secondary structure is depicted as helix and loop cartoons. Putative phosphorylation sites are highlighted in yellow and red, and N glycosylation sites are indicated in green.

A program called AlphaFold, an artificial intelligence (AI) system developed by the company DeepMind predicts the three-dimensional structure of proteins based on their amino acid sequence, evolutionary data and deep learning from established protein folds. Alphafold has drawn a lot of interest because it predicts protein structures with remarkable precision. Nevertheless, protein structures must be experimentally validated. To establish the 3D structure of proteins, experimental methods like X-ray crystallography, NMR spectroscopy, and electron microscopy are commonly employed.

Fetuin-A has conformational flexibility, especially in the CTR domain, which makes it near impossible to generate crystals of sufficient size and quality.
Therefore, we choose cryo-electron microscopy (Cryo-EM) instead of X-Ray crystallography for structure determination of Fetuin-A. To determine the structure of Fetuin-A by cryo EM, we teamed up with expert structural biologists from the nearby Forschungszentrum Jülich , Professor Carsten Sachse and Alexandros Katranidis PhD.

Cryo-EM uses an electron microscope to capture images of proteins that have been rapidly frozen at - 180°C to retain their native structure. Several hundred 2D images are computationally grouped and superimposed for contrast enhancement, then matched and rotated to derive a full 3D structure. Figure 4 illustrates how Cryo-EM arrives at protein 3D structures.

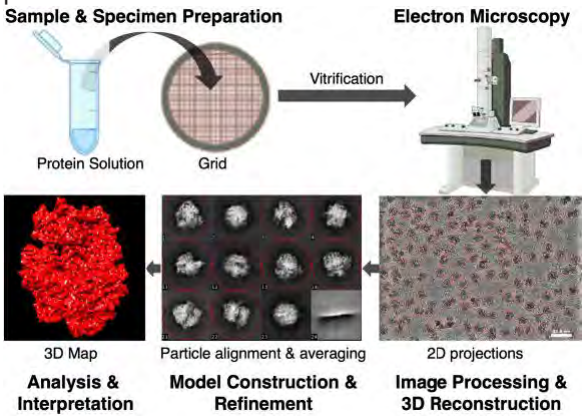

Fig. 4: Workflow of cryo-EM structure determination.
1. Sample Preparation: *To prepare a sample for cryo-EM, the quality of the sample has to be optimized by purification steps as well as adjusting the concentration.*
2. Specimen Preparation: *Ensuring that the sample is as preserved as possible prior to imaging, the sample is mounted on a cryo-EM grid and vitrified by ultrafast freezing.*
3. Image Acquisition: *Transmission electron micrographs are recorded in vacuum at ultralow temperatures maintaining the frozen state of the sample and thus an electron-dense environment.*
4. Image Processing and 3D Reconstruction: *The images are processed to create a 3D reconstruction of the sample based on the 2D projections.*
5. Model Construction and Improvement: *A model of the protein structure is constructed and iteratively improved.*
6. Analysis and Interpretation: *To comprehend the structure and function of the protein, analysis and interpretation must be done on the model.*

Fetuin-A is involved in mineral transport, it likely plays an important role in diseases involving mineral dysbalance like chronic kidney disease (CKD) and cardiovascular disease (CVD). Alterations in the metabolism of calcium and phosphate in CKD and CVD can result in pathological calcification of blood vessels and other soft tissues. Thus, understanding the molecular mechanics of Fetuin-A and its associated metabolic pathways may point to novel therapies for CKD and CVD patients.

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

Aaron Morgan, PhD candidate

To facilitate the testing of cellularized biohybrid materials and implants, current calcification testing devices needed to be miniaturized and made to be compatible with cell culture conditions. A miniaturized, dual-channel flow device was developed that mimics the flow found in arterial vessels, with one side under high flow (vessel lumen) and one side under perfusion conditions (vessel wall).

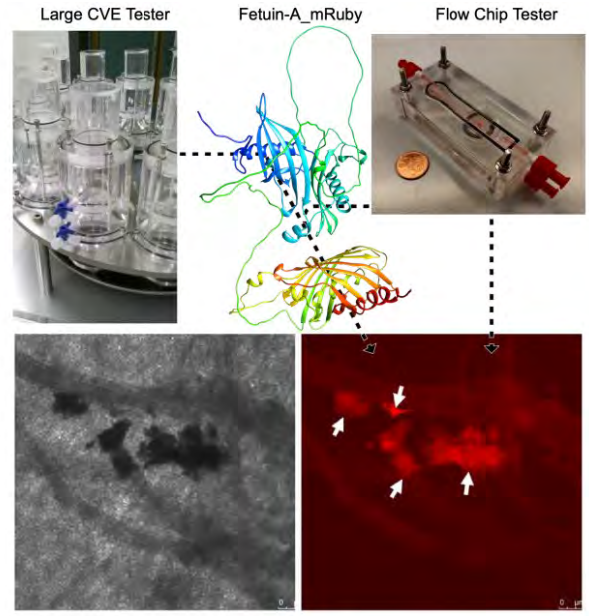

Fig. 5: Workflow of device miniaturization, using fluorescence-labelled Fetuin-A to identify calcifications in vitro.

The miniaturized flow device shown in Fig. 5 is used together with the protein Fetuin-A, a mineral chaperone found in the blood. Fetuin-A selectively binds to nascent mineral and, by attaching fluorescent labels to the protein, can be used to stain any calcifications in the sample. The transparent body of the device allows for live microscopy during experiments to monitor the progression of these calcifications. This method can be used to test the calcification propensity of materials, as well as cellularized tissue constructs.

Selected References

[1] Heitzer M, Modabber A, Zhang X, Winnand P, Zhao Q, Bläsius FM, Buhl EM, Wolf M, Neuss S, Hölzle F, Hildebrand F, Greven J. In vitro comparison of the osteogenic capability of human pulp stem cells on alloplastic, allogeneic, and xenogeneic bone scaffolds. BMC Oral Health. 2023

[2] Mert M, Malyaran H, Wolf M, Jahnen-Dechent W, Neuss S. Comparative analysis of the regenerative potential of human periodontal ligament stem cells from maxillary and mandibular molars. J Periodontol 2022

[3] Stalmann USA, Banjanin B, Snoeren IAM, Nagai JS, Leimkühler NB, Li R, Benabid A, Pritchard JE, Malyaran H, Neuss S, Bindels EM, Costa IG, Schneider RK. Single-cell analysis of cultured bone marrow stromal cells reveals high similarity to fibroblasts in situ. Exp Hematol 2022

[4] Roth CE, Craveiro RB, Niederau C, Malyaran H, Neuss S, Jankowski J, Wolf M. Mechanical Compression by Simulating Orthodontic Tooth Movement in an In Vitro Model Modulates Phosphorylation of AKT and MAPKs via TLR4 in Human Periodontal Ligament Cells. Int J Mol Sci 2022

[5] Radermacher C, Malyaran H, Bastos Craveiro R, Peglow S, Behbahani M, Pufe T, Wolf M, Neuss S. Mechanical loading on cementoblasts: A Mini Review. Osteologie 2022

[6] Knaup I, Symmank J, Bastian A, Neuss S, Pufe T, Jacobs C, Wolf M. Impact of FGF1 on human periodontal ligament fibroblast growth, osteogenic differentiation and inflammatory reaction in vitro. J Orofac Orthop 2022

[7] Rudloff S, Jahnen-Dechent W, Huynh-Do U. Tissue chaperoning - the expanded functions of Fetuin-A beyond inhibition of systemic calcification. Pflügers Archiv - Eur J Physiol 2022

[8] Ricken F, Can AD, Gräber S, Häusler M, Jahnen-Dechent W. Post-translational modifications glycosylation and phosphorylation of the major hepatic plasma protein Fetuin-A are associated with CNS inflammation in children. Plos One 2022

[9] Heuschkel MA, Babler A, Heyn J, van der Vorst EPC, Steenman M, Gesper M, Kappel BA, Magne D, Gouëffic Y, Kramann R, Jahnen-Dechent W, Marx N, Quillard T, Goettsch C. Distinct role of mitochondrial function and protein kinase C in intimal and medial calcification in vitro. Frontiers Cardiovasc Medicine 2022

[10] Jaminon AMG, Rapp N, Akbulut AC, Dzhanaev R, Reutelingsperger CP, Jahnen-Dechent W, Schurgers LJ. A Semi-Automated and Reproducible Biological-Based Method to Quantify Calcium Deposition. J Vis Exp 2022

Team

RWTH SportsDay

Gordon Conference for Biomineralization

Christmas Dinner at "Golden Pig", Aachen