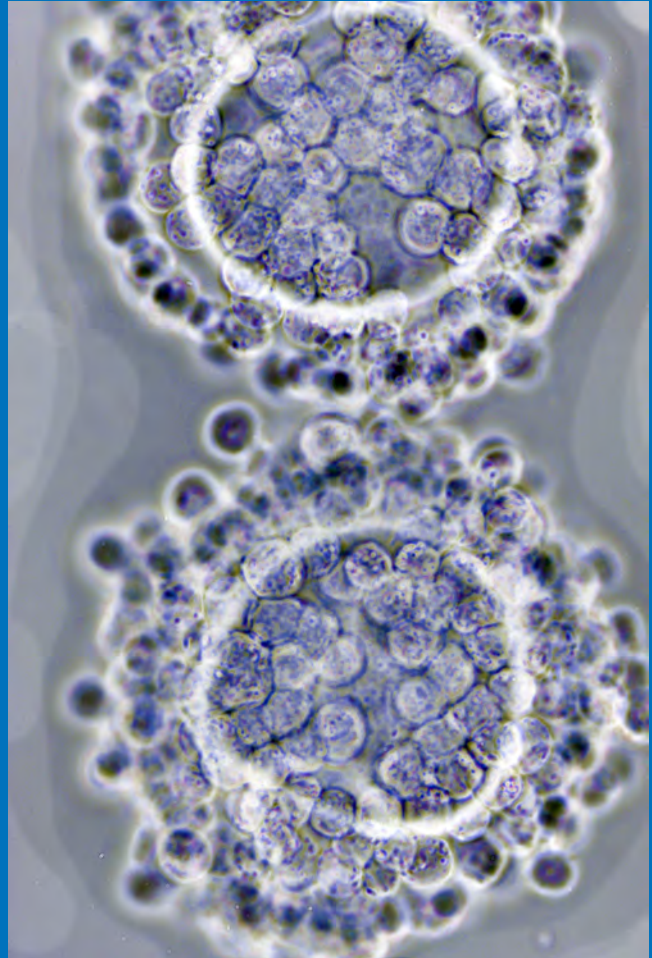
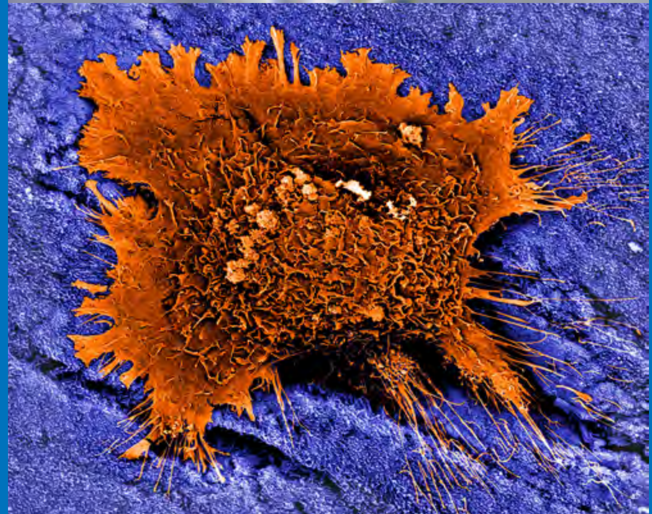
Bio interface Biointerface Laboratory at Helmholtz-Institute for Biomedical Engineering | RWTH AACHEN UNIVERSITY

Faculty of Medicine

Cell-Material Interactions: Translating Basic Science Into Clinical Applications





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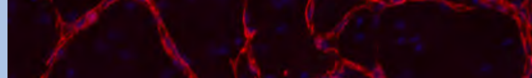


Cover Figures: Top Mouse oocytes surrounded by follicle cells; Bottom Bone marrow derived osteoclast with membrane ruffles.

Introduction

Willi Jahnen-Dechent, Professor

The year 2020 was COVID front, back and center. Like everybody else, we had a hard time coping with the restrictions imposed by the pandemic. On the positive side we adopted online conference tools in a flash, distributed precious lab time between members to minimize contact time and adopted new teaching tools including Vlogging instructions. Miraculously, and thanks to a concerted effort of all, we actually got a lot of work done without anybody getting hurt! On the negative side, we dearly miss social interactions, we long for the yearly holiday events especially our yearly lab out – makes one cherish all the things we took for granted before COVID!

In these times of reckoning we reviewed our own and others' work. „The ONE" major goal of ours in the biomineralization field is the molecular structure of the plasma protein fetuin-A and its calcium phosphate cargo. Over the past years we showed that fetuin-A is a mineral chaperone preventing growth and precipitation of calcium phosphate mineral in extracellular fluids, hence our graphic phrase „mud in the blood". If or when we will actually determine this highly complex structure is uncertain, but for starters, we made ourselves a nice picture of where we are headed.

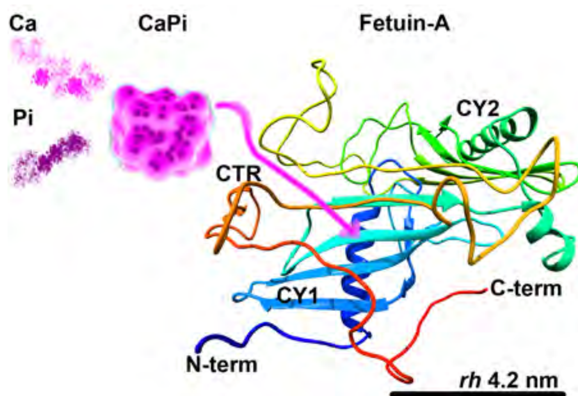


Fig. 1: Protein structure of fetuin-A.
Calcium phosphate (CaPi) binds an extended beta-sheet of the amino-terminal (N-term) cystatin-like domain of fetuin-A (CY1, blue). A second cystatin-like protein domain (CY2, green) connects to the carboxyl-terminal domain CTR (orange). CTR is intrinsically disordered and must be pried open to allow for mineral binding. rh, hydrodynamic diameter.

Oocyte Stiffness is a Quality Criteria

Carlo Schmitz, PhD

Julia Floehr, PhD

Mammalian eggs are surrounded by a protein matrix playing an important role in fertilization. Before fertilization, the protein matrix is soft and sperm can penetrate. Immediately after fertilization, this matrix is remodeled. It becomes hardened and sperm can no longer penetrate the egg. However, this matrix remodeling can also occur spontaneously before fertilization preventing the fertilization of the egg. Fertility clinics therefore require methods to assess egg quality to increase the chance of fertilization and ultimately of pregnancy.

By using nanoindentation technology we showed that fertilization-induced remodeling of the egg coat, a process called zona pellucida hardening, is indeed accompanied by an increase in the stiffness, the elastic modulus (E-modulus) of the egg coat. The coat of 2-cell embryos has an E-modulus of 454 ± 181 Pa, more than twice that of unfertilized eggs (155 ± 69 Pa). For E-modulus measurements by nanoindentation, the eggs were immobilized on a mesh and the measuring instrument, consisting of a cantilever probe, was placed in close proximity to the egg coat. The cantilever probe then indents 4 μm into the egg coat, to determine its mechanical hardness.

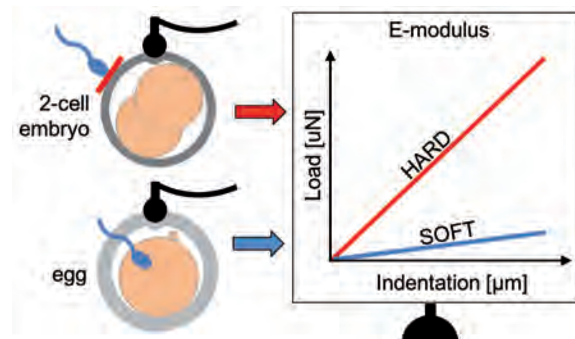


Fig.2: E-modulus measurement of eggs by nanoindentation, schematic overview.
Eggs were immobilized on a mesh and the cantilever with a spherical probe (black) of the indentation device was placed above. The cantilever probe indented 4 μm into the egg coat and measured a hardened egg coat for 2-cell embryos and a soft coat for unfertilized eggs. After hardening of the egg coat, no further sperm can penetrate the egg.

To test if mechanical indentation is suitable to assess egg quality, we used genetically modified fetuin-B deficient eggs with a predefined protein matrix structure. Those eggs have per se hardened coat that is known from eggs after fertilization – they represent fertilization failure. Using nanoindentation, we were able to clearly distinguish between the egg coat of wildtype eggs with a good quality and infertile fetuin-B deficient eggs. In numbers: compared to wildtype eggs, fetuin-B deficient eggs thus had a sevenfold higher

E-modulus (155 ± 69 Pa vs. 1104 ± 304 Pa). In conclusion, we established a method for egg quality determination that i) quantitatively measures individual egg hardness, ii) is non-destructively and iii) observer-independent.

Recombinant RANKL for Osteoclast Studies

Robert Dzhanaev, MD/PhD student

Mineralized tissues like bone can only be degraded by highly specialized cells called osteoclasts. These are giant multi-nucleated cells derived from myeloid precursors. Given the similarity of bone biomineralization and pathological calcification, we hypothesized that osteoclasts may also remove pathological calcification. Differentiation, survival and activation of osteoclasts critically depend upon receptor activator of nuclear factor κB ligand, RANKL. Together with its cell membrane receptor RANK, and the soluble decoy receptor osteoprotegerin OPG, RANKL plays a pivotal role in bone resorption. To study RANKL activation of osteoclasts, we produced recombinant RANKL in a mammalian cell line. Gel electrophoresis and immune blotting of the recombinant protein confirmed high purity of the product.

Fig. 3: Recombinant RANKL purification.
Gel electrophoresis revealed protein bands at approx. 25 kDa in the elution fractions E3-E9 (black arrowhead). Faint bands at 55 kDa in the fractions E5-E7 suggested the presence of RANKL dimers (red arrowhead). S – supernatant, FT - flow-through, W - wash fraction, DV - dead-volume column.

The biological activity of RANKL was confirmed in osteoclast cultures and bone resorption assays. Mouse bone marrow cells were treated with macrophage colony-stimulating factor (M-CSF) and commercial RANKL or homemade RANKL. The appearance of giant multinucleated cells in both RANKL-treated cultures demonstrated successful differentiation of viable osteoclasts.

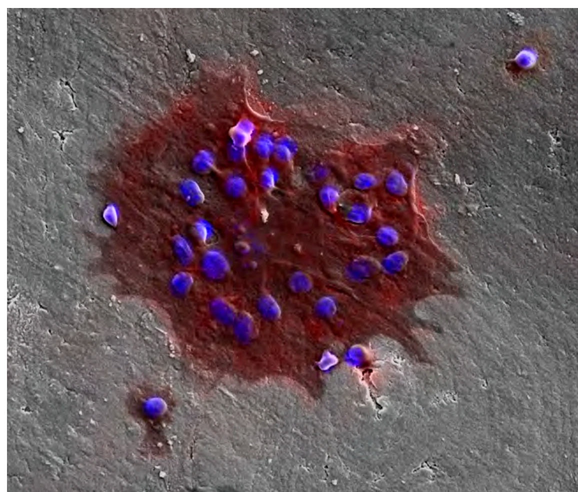


Fig. 4: SEM picture of a multi-nucleated bone marrow-derived osteoclast after 14 days of RANKL treatment.
Both commercial and homemade RANKL cytokine mediated osteoclastogenesis from bone marrow cells as indicated by the formation of multi-nucleated giant cells.

Bone resorption by differentiated osteoclasts was assessed using thin bovine cortical bone slices. Bone marrow-derived osteoclastic precursors were plated on bovine bone discs and stimulated with M-CSF and RANKL. Scanning electron microscopy confirmed the presence of multi-nucleated osteoclasts, which actively resorbed bone, forming resorption pits.

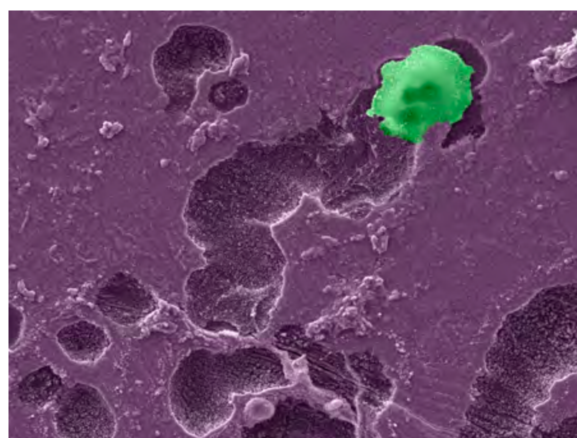
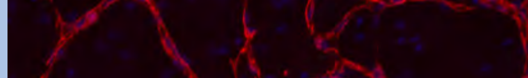


Fig. 5: SEM picture of an osteoclast on bovine bone disc.
An osteoclast (green) was observed during resorption of bone. Osteoclasts leave resorption pits and tracks while making their way through bone.

In conclusion, we produced biologically fully active RANKL that can be further developed into a theranostic agent to image and to treat pathological calcifications.



Stem Cells and Tissue Engineering

Sabine Neuß-Stein, Professor

The „Stem Cells and Tissue Engineering Group" pursued three major 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.

During the last two decades, we developed a recruitment system for endogenous MSC based on biomaterials releasing a potent chemoattractant to improve wound healing.

Together with Professor Michael Wolf, director of the Orthodontics Clinic at RWTH Aachen University Hospital, we study cellular mechanisms of PDL cells and cementoblast development, their cell-to-cell-communication and impact on wound healing, which is faster and more efficient in the upper jaw, than in the lower jaw. Hanna Malyaran reports on this work.

Together with Professor Andrij Pich, head of the research area Functional and Interactive Polymers at DWI - Leibniz Institute for Interactive Materials, we develop fibrin-based hydrogels. Depending on the kind and mixture of copolymers, a wide array of fibrin-based hydrogels form with different fiber sizes and elastic modulus, both of which are critically important in determining cell behavior. Svenja Wein presents this work in more detail.

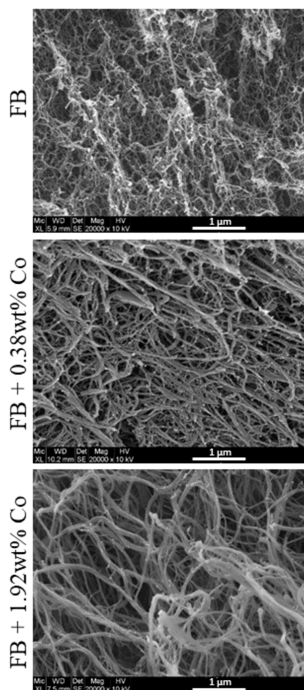


Fig. 6: Scanning electron micrographs of fibrin-based hydrogels. Fibrin-based (FB) hydrogels were synthesized with and without capolymers (Co). Increasing the copolymer concentration strongly increased fiber thickness.

Fibrin-based Hydrogels for Biohybrid Implants

Svenja Wein, PhD student

Biohybrid implants comprize a biomaterial scaffold and, ideally, the patient's own cells. Within a larger consortium of researchers, we develop fibrin gels with a textile support for biohybrid heart valve tissue engineering. We combine fibrinogen and poly(N-vinylcaprolactam) copolymers with smooth muscle cells, induced by adding TGF-β1 and BMP4 growth factors to the hydrogels. The contractile phenotype of smooth muscle cells is assessed as salient property of functional heart valve smooth muscle cells. Vascularization of the tissue engineered constructs is important. Therefore, we assess capillary formation and marker gene expression in vascular precursor cells.

Initial experiments show long-term stable hydrogels, which support the proliferation of human stem cells, so that the myogenic differentiation of the cells in the gels can be achieved in a subsequent step. The successful expression of contractile markers was demonstrated after 21 days of culture. Angiogenesis assays demonstrated formation of capillary-like structures in culture. In future we will analyze the influence of cyclic stretching on stem cell differentiation.

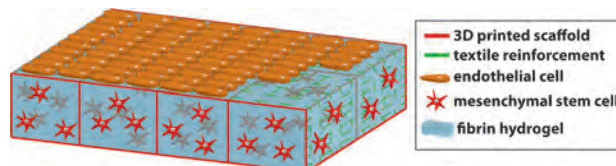


Fig. 7: Structural design of the biohybrid implant for a heart valve replacement built from a support structure coupled with the patient's own stem cells.

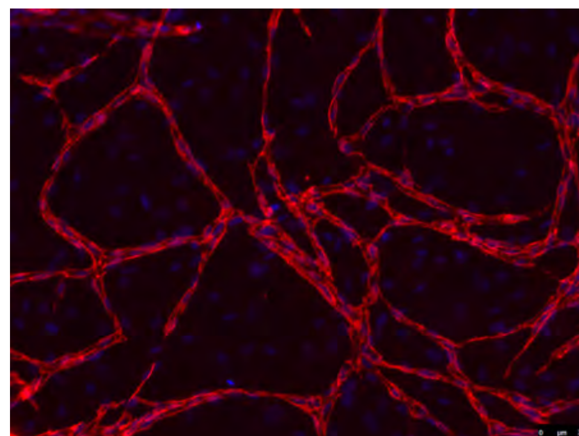


Fig. 8: Human umbilical cord vascular endothelial cells (HUVEC) form capillary networks when co-cultured with mesenchymal stem cells (MSC).

Biointerface

Impact of Stem Cells on Wound Healing and Integration of Tissue Engineered Alveolar Bone

Hanna Malyaran, PhD student

Periodontal disease usually involves recession of the periodontal ligament (PDL) ultimately leading to tooth loss from the alveolar bone. The PDL plays an important role in physiological tooth function and is important for periodontal regenerative therapy. Clinical observations indicate that wound healing and bone formation in alveolar bone varies depending on the exact localization of the lesion. Wound healing is faster in the maxilla, the upper jaw, than in the mandible, the lower jaw.
Alveolar bone differs in composition, with 23% bone marrow and 46% lamellar bone in the upper jaw, and 16% bone marrow and 63% lamellar bone in the lower jaw. The PDL hosts endogenous stem cells. In 2004, PDL precursor cells were first isolated from extracted third molars, demonstrating self-renewal and differentiation capacity towards mesodermal cell fates, and therefore referred to as stem cells. Like mesenchymal stem cells (MSC), PDL cells are now extensively studied with respect to ligament and bone formation.
We isolated PDL cells from extracted third molars of young and healthy patients. Teeth were extracted from both upper and lower jaws to allow a comparison of maxilla and mandible-derived PDL cells. MSC isolated from the spongiosa of femoral heads served as the gold standard of MSC differentiation.
Like MSC, PDL cells were positive for CD73, CD90 and CD105, and negative for CD34 and CD45. PDL cells from the upper jaw showed more proliferation and differentiation when compared to PDL cells from the lower jaw. These results support the clinical hypothesis that wound healing of the upper and the lower jaw might differ due to different cell behavior. Next, we will identify regulatory gene networks involved in periodontal cell differentiation.

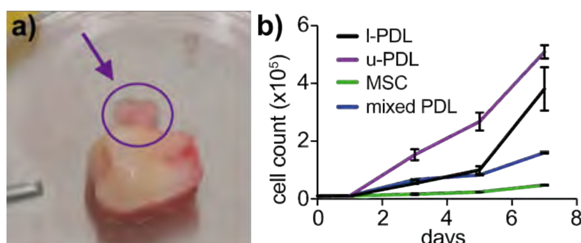


Fig. 9: Characterization of PDL cells from upper and lower jaw.
a) Periodontal tissue is scratched off the tooth with a scapel, digested in (FB) collagenase type 1 for one hour, centrifugated and seeded in a culture plate. b) PDL cells from the lower jaw (l-PDL), upper jaw (u-PDL), MSC and a mixed PDL population of 10 donors (Lonza, Cologne, DE) were seeded at a density of 5000 cells/cm^2. Proliferation rate was measured after 1, 3, 5 and 7 days.

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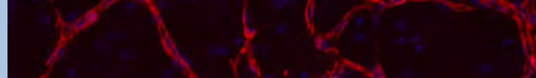

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Team in February 2021