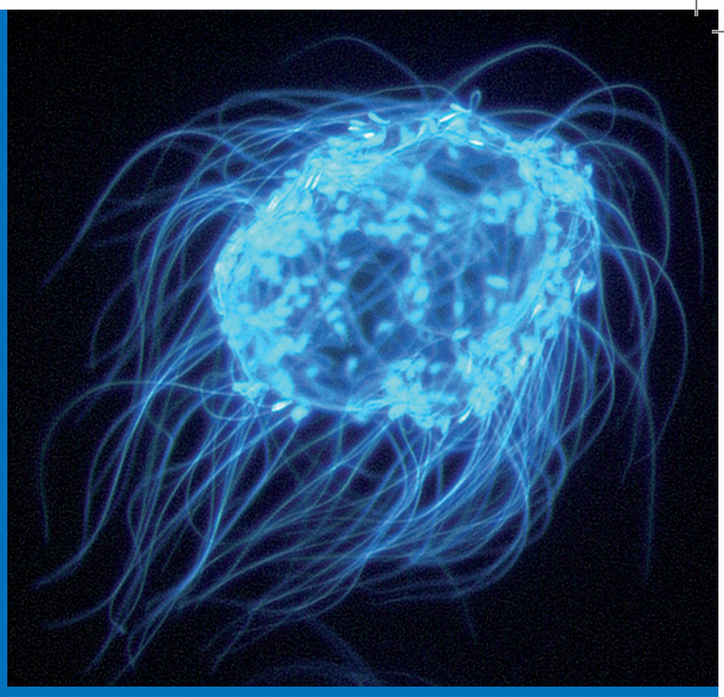
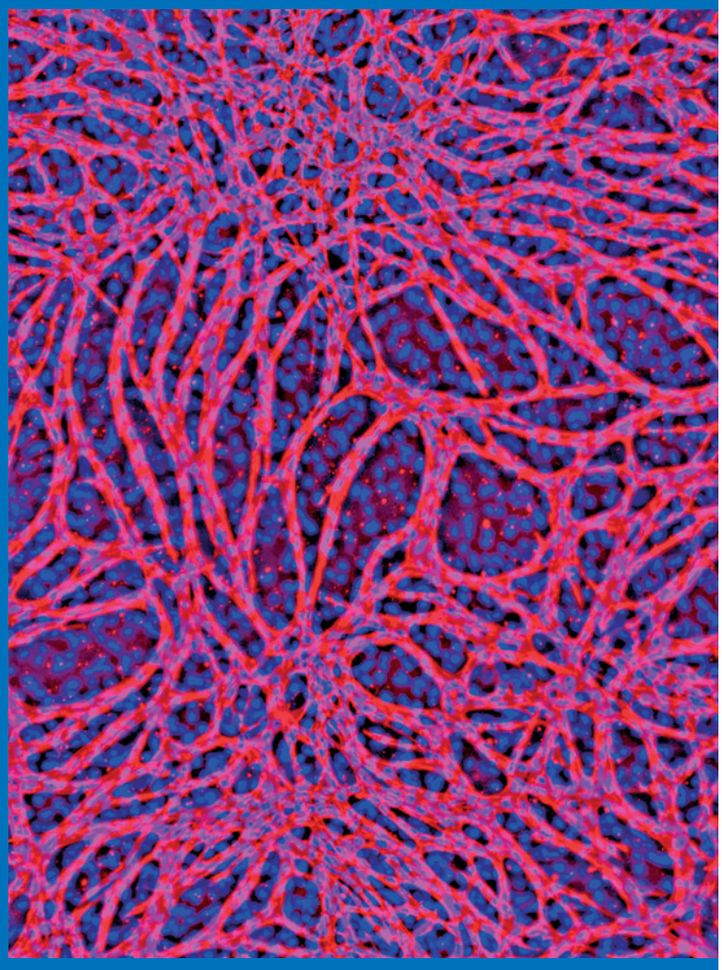
Biointerface

Faculty of Medicine

Cell-Material Interactions: Translating Basic Science Into Clinical Applications





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Title Figure Top: *In-vitro fertilization of mouse oocytes to test the influence of the plasma fetuin-B in maintaining fertility.* ***Bottom:*** *Human umbilical vein endothelial cells form capillary-like structures when co-cultured with human mesenchymal stromal cells.*

Introduction

Three PhD students successfully defended their thesis work in 2014, and seven BSc and MSc theses were completed. Thus every second member of the academic group members advanced their careers one notch - living proof that a University is half about teaching and learning. The second half is ideally spent on research, which can be challenging at times. Allocating funds now almost takes up a "third half" of our time. In 2014 we secured a prestigious individual grant from Deutsche Forschungsgemeinschaft to study the role of fetuin-B in fertilization, which is also the start of this years research report.

Fetuin-B is Essential for Fertilization

(Julia Floehr)

Previously we showed that the plasma protein fetuin-B is essential for fertilization in mice[1]. Fetuin-B is a liver-derived plasma protein that freely transfers from the blood stream to the follicular fluid. Follicular fluid surrounds the oocytes providing nutrients and signals for proper development of the oocytes. In fetuin-B deficient female mice, the complete lack of fetuin-B protein leads to premature hardening of the zona pellucida and thus to infertility. A general overview about the fetuin-B function is shown in figure 1. We now study if fetuin-B also plays a role in idiopathic infertility in women. Fetuin-B is well conserved in mammals; mouse and human fetuin-B have 68 % amino acid sequence identity and 77 % homologous amino acid exchanges suggesting structure and function conservation between species.

I developed a sandwich ELISA to measure the fetuin-B concentration in human serum. The human fetuin-B serum level averaged 0.005 g/l and thus was much lower than mouse serum fetuin-B (0.15 g/l). Complete fetuin-B deficiency like in the knockout mice was never observed. No obvious differences were detected between sexes. Studies in healthy women showed that fetuin-B is expressed fairly constant throughout the estrous cycle. No obvious association linked serum fetuin-B to hormones LH or progesterone, or to age. Genetic mutations in the FETUB gene may nevertheless cause a non-functional form of fetuin-B. Studies analyzing putative fetuin-B gene mutations are ongoing.

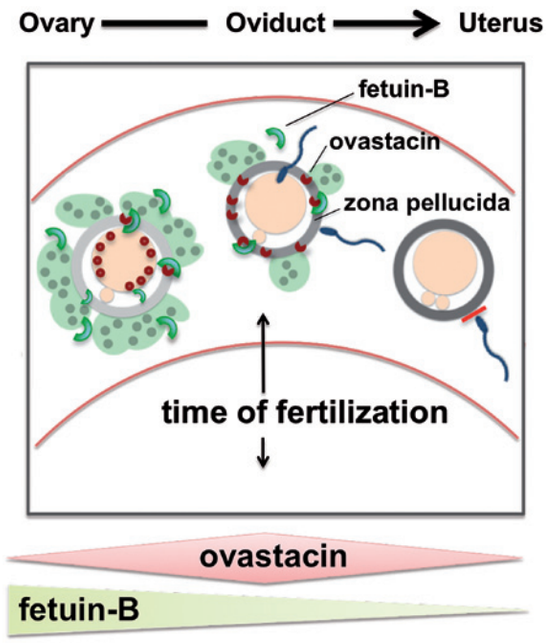

Fig.1: Fetuin-B is essential for fertilization. Fertilization triggers hardening of the zona pellucida, the outer shell of mammalian oocytes. This prevents further sperm binding and penetration, and protects the pre-implantation embryo. The hepatic plasma protein fetuin-B inhibits ovastacin, an oocyte protease mediating definitive zona pellucida hardening before fertilization. Fetuin-B thus maintains female fertility.

Nevertheless further studies of IVF patient gave first indications that fetuin-B could be useful as a predictive marker for IVF success. The results of our investigations showed that fetuin-B could be an important target in the field of reproductive biology. An increase of the fertility success would reduce the mental stress, the hormonal treatment and costs of the so far childless couples.

Therapy of Soft Tissue Calcification in Fetuin-A Deficient Mice

(Anne Babler)

Like fetuin-B, fetuin-A is a hepatic serum protein. Unlike fetuin-B, which regulates fertility, at least in mice, fetuin-A plays a major role in mineralized matrix metabolism. It acts as a mineral chaperone mediating the stabilization of protein-mineral complexes and their clearance from circulation. Fetuin-A deficient mice on the genetic background DBA/2 post-natally develop severe soft tissue calcification particularly in highly capillarized and metabolically active tissues.

Using DBA/2 fetuin-A deficient mice as a disease model, we tested various therapies to stop calcification progression. Excess dietary phosphate is a known risk factor for calcification, while fetuin-A itself and dietary magnesium should reduce calcification.

Soft tissue calcification in the mice was scored *in vivo* by computed tomography (CT) and fluorescence molecular tomography (FMT) employing labeled fetuin-A as a molecular probe for calcified matrix (fig. 2). Post mortem analysis included chemical calcium quantification, and histochemistry of tissue sections.

Using this approach we could demonstrate favorable outcomes for various dietary and pharmacological interventions in the calcification-prone DBA/2 fetuin-A deficient mice.

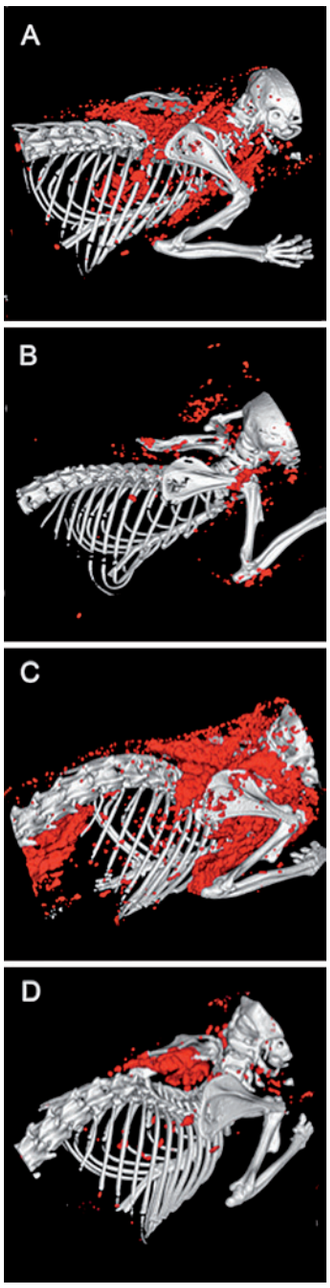

Fig. 2: Computed tomography of DBA/2 fetuin-A deficient mice treated with magnesium or phosphate. *Fetuin-A deficient mice received either control food A) or high magnesium B) or high phosphate C) or low phosphate D) food. Computed tomography of the mice was performed after a feeding period of eight weeks. Calcification was segmented (red color). Mice treated with magnesium B) had less calcification than control animals A). Elevated dietary phosphate also increased the amount of calcification C) whereas reduced phosphate intake resulted in less calcification D).*

Role of Plasma Protein Fetuin-A in Longitudinal Bone Growth

(Laura Brylka)

Fetuin-A (Ahsg) is the most abundant non-collagenous protein in bone. Therefore it is not surprising that fetuin-A deficient animals show abnormalities in their bone phenotype. Bone mineralization is normal but fetuin-A deficient mice have shorter proximal hind limbs, as well as disordered growth plates in all long bones (fig. 3). This indicates that fetuin-A plays a role in the longitudinal growth of bones, a process that occurs through endochondral ossification.

Endochondral ossification takes place in the growth plates of all long bones. The growth plate is spatially and temporally divided into different zones containing chondrocytes of various maturation stages. Chondrocytes from the so-called resting zone start proliferating and produce characteristic columnar cell stacks. Next, chondrocytes synthesize extracellular matrix and undergo hypertrophy before they ultimately start to mineralize their extracellular matrix. Following this, the chondrocytes die. Next cartilage is replaced by bone through enzymatic degradation of mineralized matrix through chondro- and osteoclasts and deposition of bone by osteoblasts. Matrix metalloproteinases 9 and 13 (MMP9, 13) mediate the tissue degradation, and vascular endothelial growth factor (VEGF) secreted by hypertrophic chondrocytes concomitantly triggers angiogenesis, and facilitates the invasion of growing blood vessels. Defects in one of these components leads to abnormal bone growth, so-called chondrodysplasia, and thus to skeletal defects.

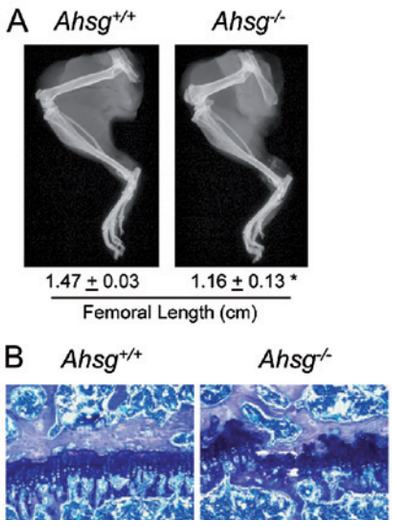

Fig. 3: Bone phenotype abnormalities in fetuin-A deficient mice. *A) Contact radiograph of the hindlegs from 4-month-old Ahsg+/+ and Ahsg-/- mice. Length of the femoral bones is given below. B) Toluidin blue staining of tibial growth plates. Ahsg -/- mice showed pronounced discontinuities in the chondrocyte column organization in comparison to the wildtype mice.*

The aim of my study is to define the molecular role of fetuin-A in the context of endochondral ossification. To this end, I investigate newborn fetuin-A deficient mice and their wildtype littermates. Histological sections (fig. 4) revealed an increase in the length of the hypertrophic zone in fetuin-A deficient mice. This difference was found in littermates of both genetic backgrounds C57BL/6 and DBA/2, indicating that - unlike the calcification propensity in fetuin-A deficient mice, which is described by Anne Babler elsewhere in this report -, the function of fetuin-A in the growth plate is strain independent.

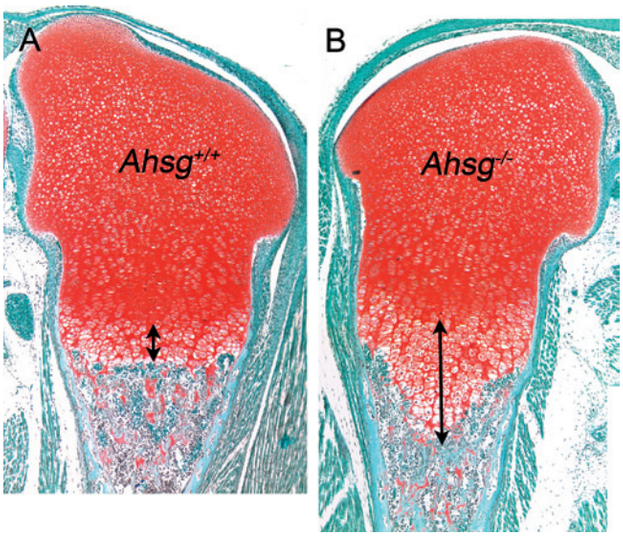

Fig. 4: Elongated hypertrophic zone in fetuin-A deficient mice. Safranin O and fast green FCF staining of the distal femur of a A) wildtype and B) fetuin-A deficient littermates. Lines with arrows indicate the length of the hypertrophic zone.

Von Kossa staining of undecalcified bone sections showed that the entire elongated growth plate in fetuin-A deficient mice is mineralized corroborating the role of fetuin-A as a regulator of mineralization (fig. 5).

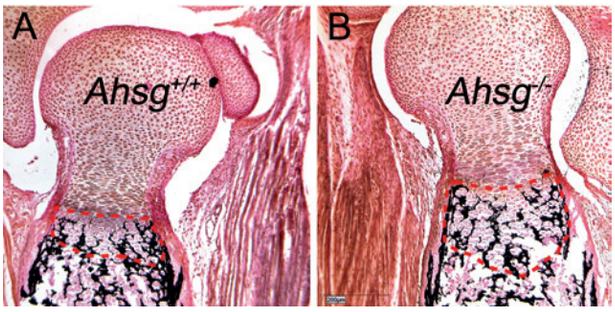

Fig. 5: Elongated hypertrophic zone in fetuin-A deficient mice is mineralized. Von Kossa staining of the proximal ulna in A) wildtype and B) fetuin-A deficient littermates. The hypertrophic zone is highlighted with a red dashed line.

Taken together, my findings show that fetuin-A plays a role in matrix remodeling during endochondral ossification and that fetuin-A might directly or indirectly influence the activity of major remodeling enzymes, leading to an increase in the length of the hypertrophic zone.

Stem Cell-Based Tissue Engineering

(Sabine Neuss-Stein)

In 2014 we focussed on two major topics: (i) *in vivo* recruitment for endogenous mesenchymal stem cells (MSC) to improve tissue regeneration and (ii) stem cell-based bone tissue engineering.
In her final PhD student year, Julia van de Kamp succeeded in developing an MSC recruitment system based on hepatocyte growth factor-loaded biomaterials. 10 years ago, we found that HGF is a potent chemoattractant for MSC (Neuss et al., Stem Cells, 2004). Now we incorporated HGF into collagen and fibrin gels as well as in silk fibers. HGF released from these biomaterials attracted MSC *in vitro* (van de Kamp, Stem Cells International, 2013), as well as *in vivo*. After subcutaneous implantation of HGF-loaded biomaterials in mice, more MSC were detected in the tissue surrounding HGF-loaded biomaterials than around HGF-free biomaterials. Julia finished her PhD work in November and has then moved overseas. We wish her all the best and continued success in all endeavors.
To improve bone tissue engineering, we used complex three-dimensional (3D) co-cultures in bioreactors to improve osteogenic differentiation of MSC over traditional static culture on flat biomaterials. In cooperation with Professor Fischer's group at the Department of Dental Materials and Biomedical Research (ZWBF) and with clinical partners in Orthopedics, Cranio-Maxillofacial Surgery, as well as with Spintec-Engineering GmbH we studied a variety of 3D materials, including slip-casted β-TCP and ceramics, electrospun poly-Caprolacton, PDLLA and PEEK, and silk non-wovens. These 3D scaffolds were either unmodified or loaded with factors playing a role in osteogenesis, cell adhesion or stem cell recruitment. Osteogenesis and angiogenesis are tightly orchestrated in bone regeneration. Therefore we established co-cultures of MSC and endothelial cells demonstrating that under co-culture conditions, osteogenic differentiation of MSC started earlier and was more vigorous than MSC monoculture. We used perfusion reactors and mechanoreactors to enhance osteogenic differentiation via mechanotransduction. By applying cyclic forces to stem cell/biomaterial hybrids, we successfully induced osteogenic differentiation of MSC even in the absence of osteogenic induction factors. Finally, first animal studies were performed to prove functionality of our 3D bone constructs. Here we implanted tissue-engineered bone into critical size defects in mouse femora (with the Institute of Anatomy) and sheep skull (with Cranio-Maxillofacial Surgery). These experiments are now completed and are currently being analyzed.

Biofunctionalization of Strontium-Dotated β-Tricalcium Phosphate Scaffolds with Human Mesenchymal Stem Cells and Human Umbilical Vein Endothelial Cells

(Michaela Bienert)

Demographic change and the aging population increase the demand for bone replacement materials augmenting osteoporotic fracture healing. Osteoporosis is both characterized by decreased bone mass and density. Beta tricalcium phosphate ceramics (ß-TCP) are osteoconductive and biocompatible materials in clinical use to restore lost bone structure. These materials generally show low fracture resistance, yet offer a chemical environment facilitating new bone formation. ß-TCP is readily modified to mimic cancellous bone. Figure 6 shows this material with macropores resembling bone channels. ß-TCP is biodegradable, but not osteoconductive. Strontium dotation was used to increase the osteoconductive material properties. Strontium is known to enhance osteogenic differentiation of mesenchymal stem cells (MSC) by activating Wnt/beta-catenin signaling.
Osteogenic differentiation of MSC was studied alone and in co-culture with human umbilical vein endothelial cells (HUVEC) grown on Sr-dotated ß-TCP provided by Prof. Fischer's group at RWTH Aachen University Clinic, Dental Materials and Biomaterial Research Department (ZWBF). Figure 6B shows that co-culture with HUVEC enhanced osteogenic MSC differentiation.

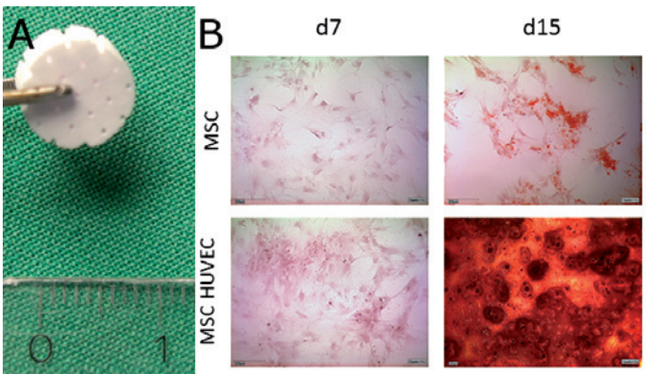

Fig. 6: β-tricalcium phosphate scaffold and osteogenic differentiation of MSC alone and in co-culture with HUVEC. *A) β-TCP ceramic plug with macropores formed by sintering and drilling (provided by ZWBF. B) Alizarin red staining of MSC monoculture in comparison to MSC in co-culture with HUVEC. Calcium mineral stained red. Cultures were kept for 7 and 15 days.*

Vascularization is an essential part of tissue engineering including bone tissue engineering. Capillary vessel formation is thought to increase bone formation in artificial scaffolds. Figure 7 shows that a feeder layer made of MSC better supported capillary formation by HUVEC than a feeder layer of dermal fibroblasts (dFB), the cell type commonly used in this kind of angiogenesis assay.

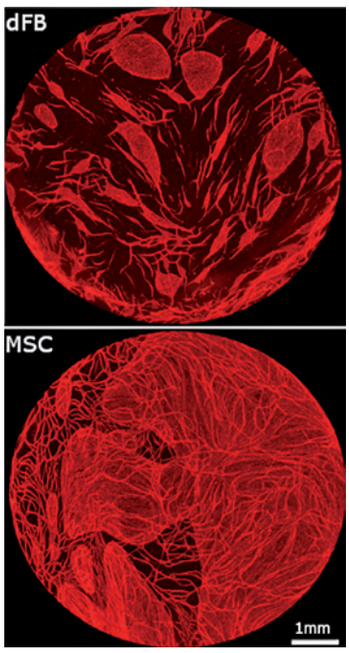

Fig. 7: Angiogenesis assay of MSC and HUVEC co-culture. *HUVEC and MSC are seeded on tissue culture plastic for 14 days. HUVEC (CD31 - red) form a highly branched network of capillary-like structures.*

Co-culture of MSC and HUVEC was also studied in ß-TCP scaffolds. To visualize cell growth inside the scaffold, Two-photon microscopy was used. Figure 8 illustrates vigorous growth of MSC and HUVEC inside a 300 μm diameter macropore within a ß-TCP scaffold.

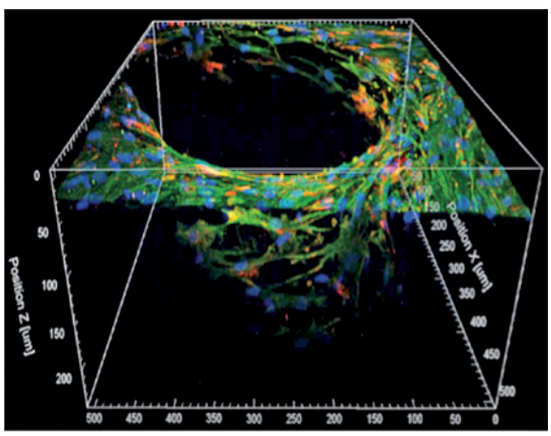

Fig. 8: MSC and HUVEC growth inside a macropore. *Co-culture is seeded on a $Ca_3(PO4)_2$ scaffold for 15 days. Two-photon microscopy: MSC+HUVEC (Vimentin-green), HUVEC (CD31-yellow), DAPI (blue). Penetration depth: ~150 μm.*

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