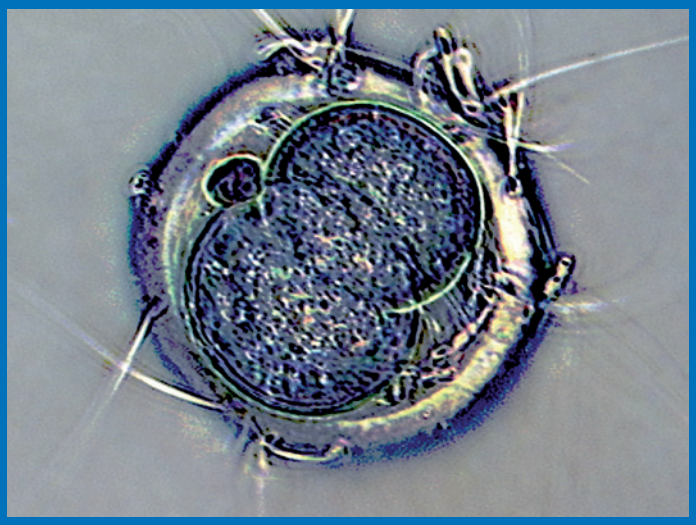
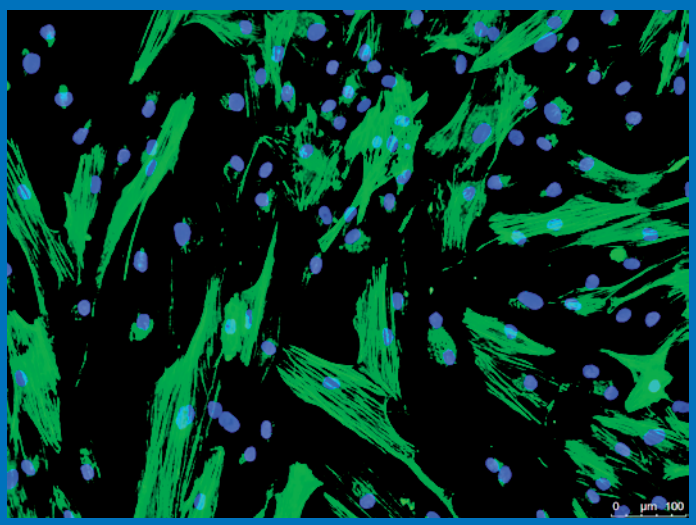
Biointerface Laboratory at Helmholtz-Institute for Biomedical Engineering

RWTH AACHEN UNIVERSITY

Faculty of Medicine

Cell-Material Interactions: Translating Basic Science Into Clinical Applications





Director

Univ.-Prof. Dr. rer. nat.
Wilhelm Jahnen-Dechent

RWTH Aachen University Hospital
Pauwelsstrasse 30, 52074 Aachen

Helmholtz-Institute for Biomedical Engineering
Pauwelsstrasse 20, 52074 Aachen

Phone: +49 (0)241 80-80157 (Secretary)
+49 (0)241 80-80163 (Office)
Fax: +49 (0)241 80-82573
Email: rsous@ukaachen.de
Web: http://www.biointerface.rwth-aachen.de

Staff

Sous, Renate Administrative Assistant
Adamzyk, Carina Dr. rer. nat.
Babler, Anne Dr. rer. nat.
Bienert, Michaela MSc
Biermann, Robin
Bruhns, Florian
Brylka, Laura MSc
Büscher, Andrea BSc
Can, Damla cand med
Carvalho Lopes Barros Menezes, Clara BSc
Da Silva Carmo, Ana BSc
Dietzel, Eileen Dr. rer. nat.
Ernst, Sabrina MSc
Floehr, Julia MSc
Gräber, Steffen CTA
Hagel, Marc-Daniel BSc
Köppert, Sina BSc
Labude, Norina MTA
Müller, Katrin BSc
Neuß-Stein, Sabine PD Dr. rer. nat.
Reinhold, Stefan BSc
Ricken, Frederik cand med
Schleypen, Tessa BSc
Schmitz, Carlo MSc
Schwarz, Miriam cand med
Wölfel, Eva Maria BSc
Weis, Daniel Dr.
Wojtasik, Magdalena BSc

Title Figures: The unsung heroes of much of our work taking mutual interest (top), in vitro fertilized mouse oocyte (center) and adipose tissue-derived stem cells differentiated towards smooth muscle cells.

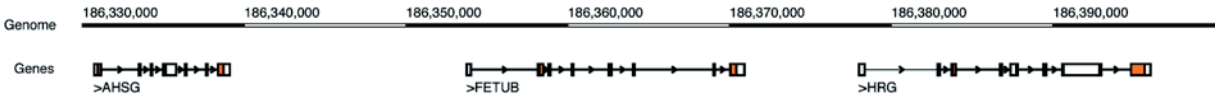


Fig. 1: Fetuin family locus on chromosome 3 of the human genome. *Fetuin-A (genetic symbol AHSG), Fetuin-B (FETUB) and Histidine-Rich Glycoprotein (HRG) are next neighbours in all published mammalian genomes demonstrating a common phylogeny of this small group of secreted liver proteins. We have knocked out these genes in mice to study their function.*

Introduction

The title page features the renewed Biointerface logo following RWTH Aachen University corporate identity. We are entitled to the logo due to our commitment and affiliation with the Helmholtz-Institute for Biomedical Engineering, the Department of Biology within the Faculty of Mathematics, Computer Science and Natural Sciences, and the Faculty of Medicine hosted by RWTH University Clinics. Translating basic natural science into clinical applications comes natural in this setting, especially in the strong engineering environment of RWTH Aachen University. Our patented invention »CalciNeph: a Blood Test For The Diagnosis of Calcification Propensity« was shortlisted as »Progress through Translation« in the 2015 University Competition »ZukunftErfindenNRW«, announced by Svenja Schulze, the state minister for innovation, science and research.

Fig. 2: Prize Ceremony *for the State University Competition "ZukunftErfindenNRW", which took place at Zeiss Planetarium Bochum, September 2015. From left Svenja Schulze (State Minister), Beate Kowollik (WDR), Willi Jahnen-Dechent.*

Another highlight of the year was the PhD degree »with distinction« awarded to Carina Adamzyk. Carina has now left the group for a post-doctoral stint abroad. We wish her the best of luck and a happy return. In addition, Sabrina Ernst, Dominic Laaf, Carlo Schmitz, and Magdalena Wojtasik graduated as MSc. Congratulations!

We continue our basic science program on the biological role of fetuin proteins, and our applied line of research on cell-biomaterial interactions, stem cell biology and tissue engineering. Fetuins are typical mosaic proteins stitched together from tried-and-true protein building blocks by exon shuffling (Fig. 1). Fetuin-A (genetic symbol Ahsg or Fetua), fetuin-B (Fetub), and histidine-rich glycoprotein (Hrg) are tripartite (carrying three independent protein folding domains) plasma proteins made in the liver. Two of three folding domains are closely related to cystatins. The structural homology of two out of three folding domains thus identifies fetuins as members of the cystatin superfamily of proteins. Cystatins are inhibitors of lysosomal proteinases, (enzymes from a special subunit of the cell that break down proteins) and important extracellular inhibitors of cysteine proteases. Published work however, suggests that the fetuins are physiologically silent as cysteine proteinase inhibitors, but act as serine or metalloproteinase inhibitors instead[10]. The structural basis for this somewhat surprising swap in inhibitor specificity is currently unknown. Nevertheless the cystatin folds form a stable protein backbone of all fetuins, to which bits and pieces are added or subtracted until an advantageous selectable trait has evolved. A third domain in each of the proteins is unique to each protein and carries no obvious resemblance to known secreted proteins. One could expect that fetuin-A, fetuin-B and Hrg have similar functions given their common genetic origin. In fact the experimentally verified functions are quite diverse. Fetuin-A has a major role in mineralized matrix biology and pathological calcification[8], fetuin-B regulates female fertility[6], and Hrg is involved in hemostasis[11], innate immunology[1] and angiogenesis.

Recent research employing our Hrg knockout (*Hrg*$^{-/-}$) mice has led to another surprising turn in the pleiotropic functions of fetuin proteins. *Hrg*$^{-/-}$ mice were intrinsically less prone to inflammatory liver disease because macrophages in these animals reacted more anti-inflammatory or M2-polarized in immunological terms than wildtype mice[1]. Hrg promoted M1 features of hepatic macrophages, which protected *Hrg*$^{-/-}$ mice in two chronic liver disease models from hepatic injury and fibrosis. Moreover, *Hrg*$^{-/-}$ mice showed significantly enhanced hepatic vascularization, corroborating proangiogenic activities of M2-polarized liver macrophages. Thus liver-derived HRG is an endogenous molecular factor promoting the polarization of hepatic macrophages towards the M1 phenotype, thereby accentuating chronic liver injury and fibrosis progression, but limiting angiogenesis.

Increasing *In Vitro* Fertilization (IVF) Rate with Fetuin-B Supplementation

Dr. Eileen Dietzel

Studying fetuin-B deficient (*Fetub*$^{-/-}$) mice, we determined that fetuin-B is essential for fertilization. Fig. 3 illustrates the mechanism of ovastacin inhibition by fetuin-B. Ovastacin is a metalloproteinase, which is exclusively expressed in oocytes

and is stored in small vesicles beneath the plasma membrane. Fetuin-B is a liver-derived plasma protein, which impregnates the oocyte extracellular matrix, called *zona pellucida* (ZP). For fertilization, sperm must penetrate the ZP before oocyte-sperm fusion can occur.

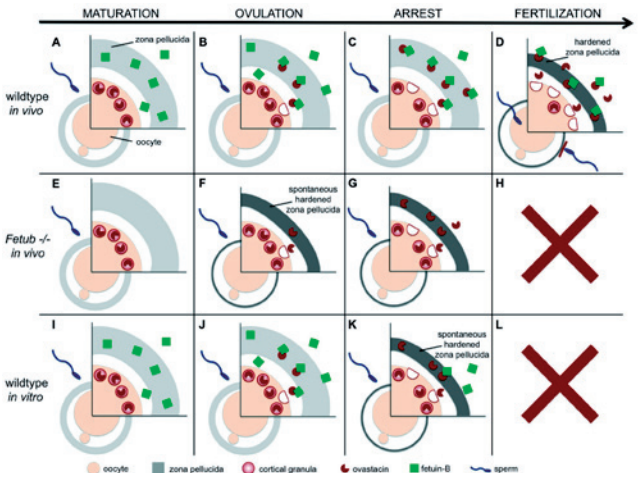


Fig. 3: Illustration of the mechanism of ovastacin and fetuin-B interaction *at the zona pellucida (ZP) in wildtype oocytes (A-D), fetuin-B deficient (Fetub$^{-/-}$) mice (E-H) and in wildtype oocytes under in vitro fertilization (I-L).*

Small amounts of ovastacin are released by the oocyte during ovulation and meiotic M2 arrest. This spuriously released ovastacin, which is inhibited by fetuin-B in wildtype mice (Fig. 3 B, C) causes premature ZP hardening (ZPH), a process that is naturally triggered after fertilization, when large amounts of ovastacin are released (Fig. 3 D). ZP hardening blocks further sperm binding and prevents polyspermy.
In the *Fetub$^{-/-}$* mice the small amounts of released ovastacin trigger spontaneous premature ZPH (Fig. 3 F). Thus fertilization is blocked and *Fetub$^{-/-}$* females are infertile.
ZPH is also a common complication of IVF. We now test if added fetuin-B protein can improve the *in vitro* fertilization rate.

Association of High Fetuin-B Concentrations in Serum with Fertilization Rate in IVF

MSc Julia Floehr

Fetuin-B is essential for fertilization in mice. Fetuin-B deficiency causes female infertility. The fetuin-B gene is well conserved between mice and humans. We studied the role of fetuin-B in human female reproductive biology and determined serum and follicular fluid fetuin-B levels in healthy volunteers and in patients attending the UKA infertility clinic (directed by Prof. Joseph Neulen).

Male and female serum fetuin-B levels in healthy volunteers remained constant over the course of one month. In females, serum fetuin-B was unaffected by the menstrual cycle (Fig. 4 A) and its associated hormonal changes. Ethinyl estradiol in healthy female volunteers on hormonal contraception, and very high estradiol levels exceeding 600 pg/ml (Fig. 4 B), attained in patients undergoing hormonal stimulation, were associated with increased serum fetuin-B. This suggested an estrogen-mediated regulation of hepatic fetuin-B expression.

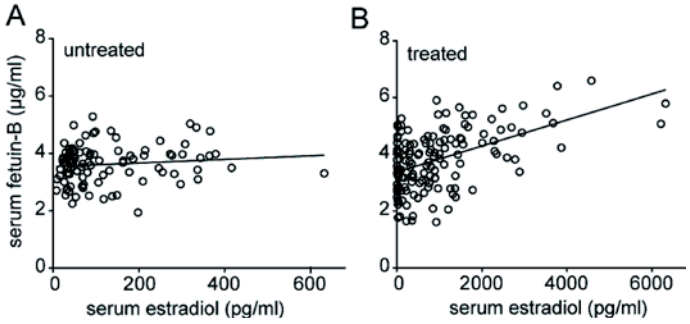


Fig. 4: Association of serum fetuin-B and serum estradiol *in women during menstrual cycle and in women undergoing hormonal ovarian stimulation. Each data point represents matched serum fetuin-B and estradiol concentrations measured during spontaneous menstrual cycle of untreated individuals (n=99, 7 individuals) and during treatment cycles of fertility clinic patients (n=142, 25 individuals). The solid lines depict Spearman linear regression (A) r=0.176; r2=0.015; p=0.08; B) r=0.414; r2=0.249; p<0.0001).*

Further we showed that serum fetuin-B increase was associated with improved IVF rate (Fig. 5 A), while fetuin-B levels remained unchanged in patients with fertilization failure (Fig. 5 B). Serum estradiol increased in both groups indicating an ovarian response to controlled ovarian stimulation (Fig. 5 C, D).

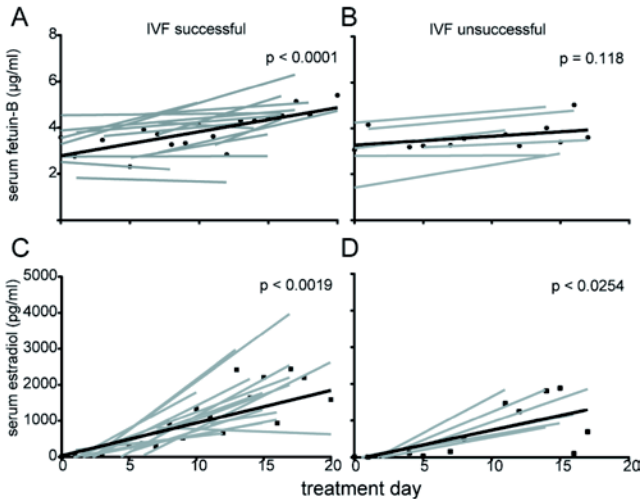
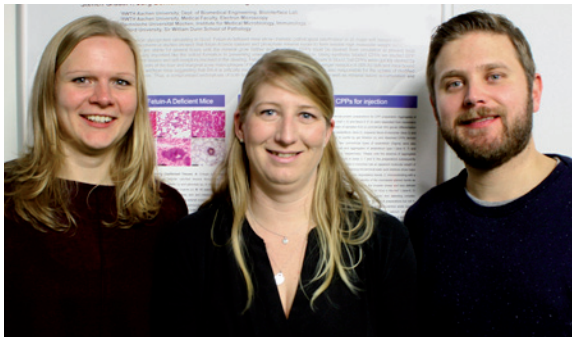


Fig. 5: Association of serum fetuin-B and IVF rate. *Serum fetuin-B increased upon ovarian stimulation in successful (at least one oocyte was fertilized) IVF cycles (n=15, p<0.0001), but remained unchanged in unsuccessful (no oocyte fertilized) IVF cycles (n=6, p=0.118). Endogenous estradiol increased in both successful and unsuccessful IVF cycles. Gray lines represent individual IVF cycles. Black lines depict the linear regression curves of mean serum fetuin-B and estradiol, respectively.*

Calciprotein Particles



BSc Andrea Büscher, Dr. Anne Babler, Dr. Daniel Weis

Calciprotein particles, in short CPPs, are hypothetical structures mediating the inhibition of basic calcium phosphate crystal formation to prevent ectopic calcification in the body.
CPPs undergo a typical Ostwald ripening process. In the first state, called primary CPPs, they reach a diameter of 50 to 80 nm. After structural rearrangements they can grow up to 500 nm and are then called secondary CPPs. Both CPPs are colloidal particles consisting of an amorphous phase of calcium phosphate and fetuin-A. After the transformation to secondary CPPs the calcium phosphate becomes more crystalline octacalciumphosphate (OCP) surrounded by a monolayer of fetuin-A and serum proteins like albumin. The secondary CPPs are stable and can be readily cleared from circulation.
We now study the ability of CPPs to induce inflammation and extracellular calcification in cell culture. Moreover, we study, if CPP clearance from the body is compromized in renal disease. The isolation of CPPs from serum has never been convincingly demonstrated. Thus we develop methods to study CPPs presence and amount in biological fluids.

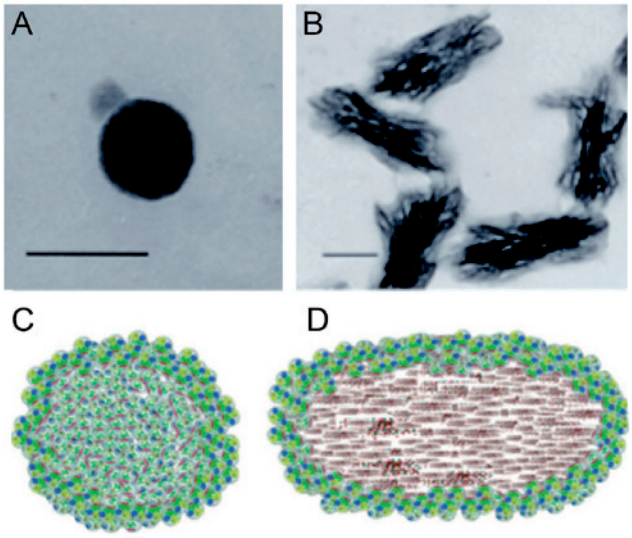


***Fig. 6: A and C depict CPPs** in their primary state, in B and D secondary CPPs are shown (modified from Jahnen-Dechent et al., 2011).*

Stem Cells and Tissue Engineering

PD Dr. Sabine Neuß-Stein

In 2015 members of the stem cells and tissue engineering lab studied in stem cell / endothelial cell interactions, mechanical forces and their influence on stem cell differentiation, xeno-free culture conditions for clinical translation and nanogel implant coatings. The »Nanocoating« project, was jointly performed with Georg Conrads (Oral Microbiology and Immunology, UKA) and Andrij Pich (DWI Leibniz-Institut für Interaktive Materialien). Seed funding came from RWTH Aachen. Nanogels were cell adhesive, yet microbe repellent. The nanogels were PEG based, and contained Isoeugenol®, which is both anti-inflammatory and antimicrobial. Nanogels proved antimicrobial against pathogenic microorganisms. We studied cell adherence, viability and proliferation of L929 mouse fibroblasts, human dermal fibroblasts (dFB) and human mesenchymal stem cells (MSC) on different nanogels (Fig. 7).

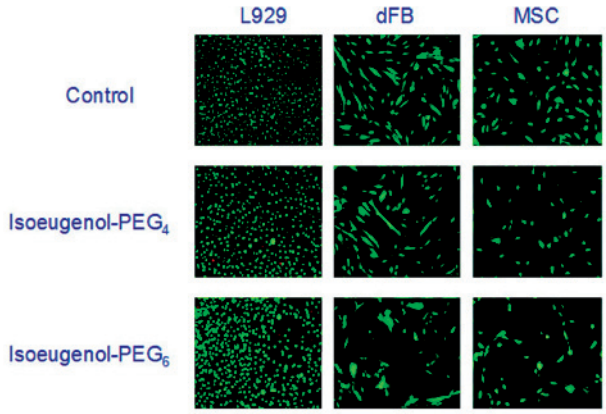


***Fig. 7: Live dead staining** with fluoresceindiacetate and propidiumiodide of L929 mouse fibroblasts, dFB and MSC on tissue culture plastic as control and on two exemplary nanogels for implant coatings. Viable cells are green fluorescent, dead cells should be red fluorescent.*

All tested cell types adhered to nanogels, displayed typical morphology and showed high viability. In addition, mesenchymal stem cells underwent osteogenic differentiation (Fig. 8). After 21 days of culture in osteogenic induction medium, Alizarin red staining suggested successful osteogenic differentiation.

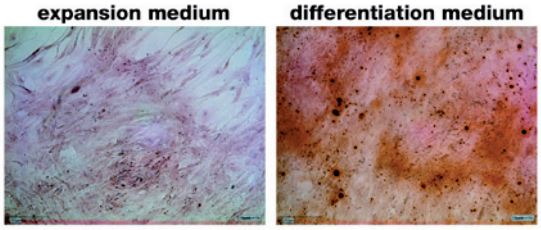


Fig. 8: Osteogenic differentiation of human mesenchymal stem cells on nanogels.

Xeno-Free Differentiation of Human Adipose-Derived Mesenchymal Stem Cells towards Smooth Muscle Cells for Tissue Engineering

MSc Michaela Bienert

Few tissue engineered constructs make it to the market because of fundamental limitations. One hurdle is the huge number of cells required to produce an autologous tissue engineered construct. Human mesenchymal stem cells derived from bone marrow (BM-MSC) are widely used for tissue engineering. However, BM-MSC have limited capacity for *in vitro* expansion. Another limitation is the use of fetal calf serum (FCS) in cell culture. A reliable procedure for MSC culture without FCS is therefor needed. Lipoaspirates are readily available for MSC isolation. In cooperation with the Tissue Engineering and Biomaterials lab of Professor Jockenhövel at the Helmholtz-Institute, we studied human adipose-derived mesenchymal stem cells (AD-MSC) isolated from lipoaspirates. The cells were expanded and differentiated without FCS. Xeno-free differentiation protocols established in our group were used to coat a small vascular graft containing smooth muscle cells.

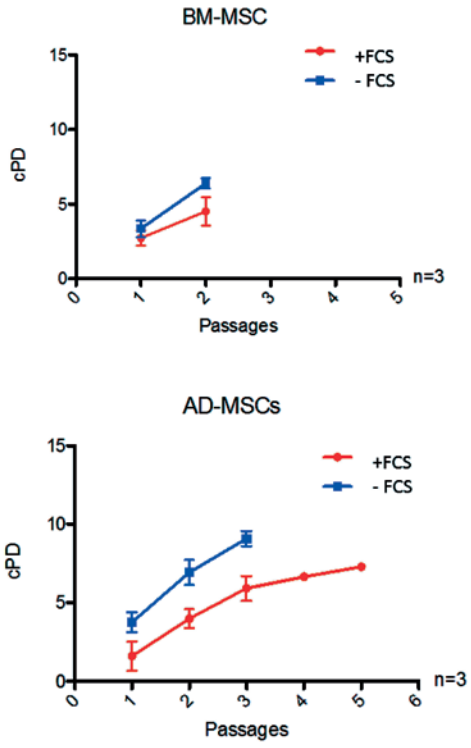


Fig. 9: Cumulative population doublings (cPD) of AD-MSC and BM-MSC.

First, the expansion of AD-MSC was characterized (Fig. 9). The cell number required for grafting a biological stent with a diameter of approximately 5 mm and 10 cm length was 10^8 cells. This number of cells was produced from AD-MSC after 5 passages with FCS, and after 3 passages without FCS. BM-MSC reached the required cell number after 2 passages with or without FCS.

Flow cytometry demonstrated that AD-MSC and BM-MSC showed a consistent phenotype over five passages (Fig. 10).

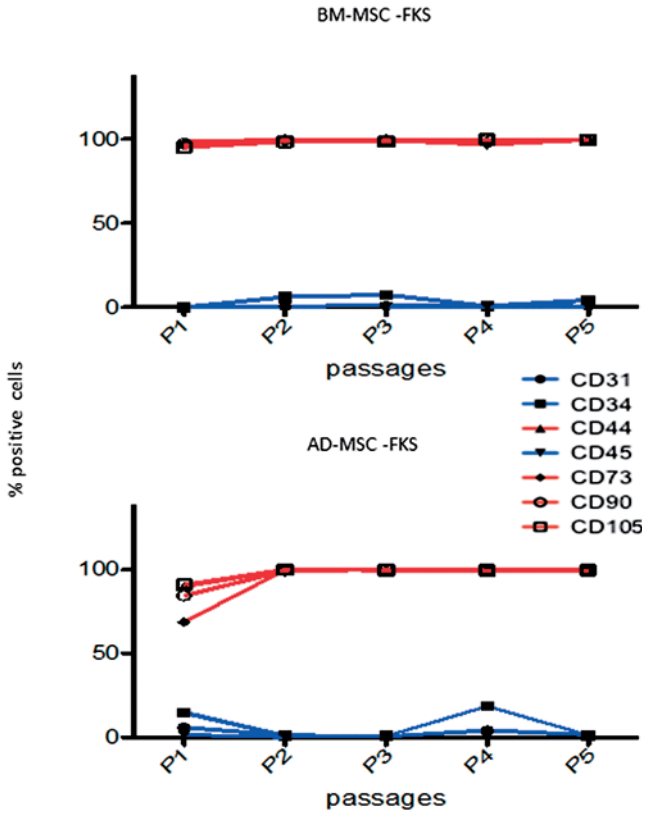


Fig. 10: Flow cytometry of AD-MSC and BM-MSC expansion. *Expansion without FCS. Passages and percentage of cells expressing representative markers are displayed. Blue lines: negative markers CD31, CD34, CD45. Red lines: positive markers CD44, CD73, CD90, CD105.*

Finally, cells were differentiated into smooth muscle cells (Fig. 11). Primary human aortic smooth muscle cells (hAoSMCs) were similar to differentiated AD-MSC with regards to the SMC markers SM22α, calponin and smooth muscle myosin heavy chain (SM-MHC).

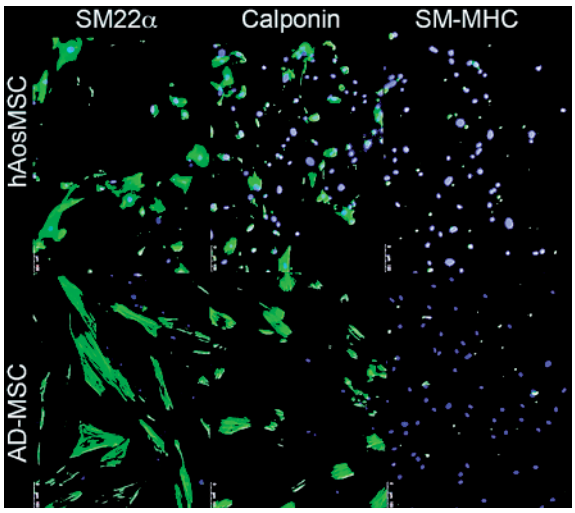


Fig. 11: Immunofluorescence of AD-MSC differentiated towards smooth muscle cells.

Selected References

[1] Bartneck M, Fech V, Ehling J, Govaere O, Theresa Warzecha K, Hittatiya K, Vucur M, Gautheron J, Luedde T, Trautwein C, Lammers T, Roskams T, Jahnen-Dechent W and Tacke F, Histidine-rich glycoprotein promotes macrophage activation and inflammation in chronic liver disease. Hepatology (Baltimore, Md), **2015**. [Epub ahead of print]

[2] Beckmann R, Lippross S, Hartz C, Tohidnezhad M, Ferreira MSV, Neuss-Stein S, Seekamp A, Nebelung S, Kweider N, Rath B, Jahr H, Pufe T and Varoga DJ, Abrasion arthroplasty increases mesenchymal stem cell content of postoperative joint effusions. BMC musculoskeletal disorders, **2015**, *16*, 250.

[3] Boda SK, Broda J, Schiefer F, Weber-Heynemann J, Hoss M, Simon U, Basu B and Jahnen-Dechent W, Cytotoxicity of ultrasmall gold nanoparticles on planktonic and biofilm encapsulated gram-positive staphylococci. Small, **2015**, *11*, 3183-3193.

[4] Dahlmann F, Biedenkopf N, Babler A, Jahnen-Dechent W, Karsten CB, Gnirß K, Schneider H, Wrensch F, O´Callaghan CA, Bertram S, Herrler G, Becker S, Pöhlmann S and Hofmann-Winkler H, Analysis of ebola virus entry into macrophages. The Journal of infectious diseases, **2015**, *212 Suppl 2*, S247-257.

[5] Duarte Campos DF, Blaeser A, Korsten A, Neuss S, Jäkel J, Vogt M and Fischer H, The stiffness and structure of three-dimensional printed hydrogels direct the differentiation of mesenchymal stromal cells toward adipogenic and osteogenic lineages. Tissue engineering Part A, **2015**, *21*, 740-756.

[6] Floehr J, Dietzel E, Neulen J, Roesing B, Weissenborn U and Jahnen-Dechent W, Association of high fetuin-B concentrations in serum with fertilization rate in IVF: A cross-sectional pilot study. Human Reproduction, **2016**, 1-11**.** [Epub ahead of print]

[7] Gremse F, Doleschel D, Zafarnia S, Babler A, Jahnen-Dechent W, Lammers T, Lederle W and Kiessling F, Hybrid μct-fmt imaging and image analysis. Journal of visualized experiments : JoVE, **2015**.

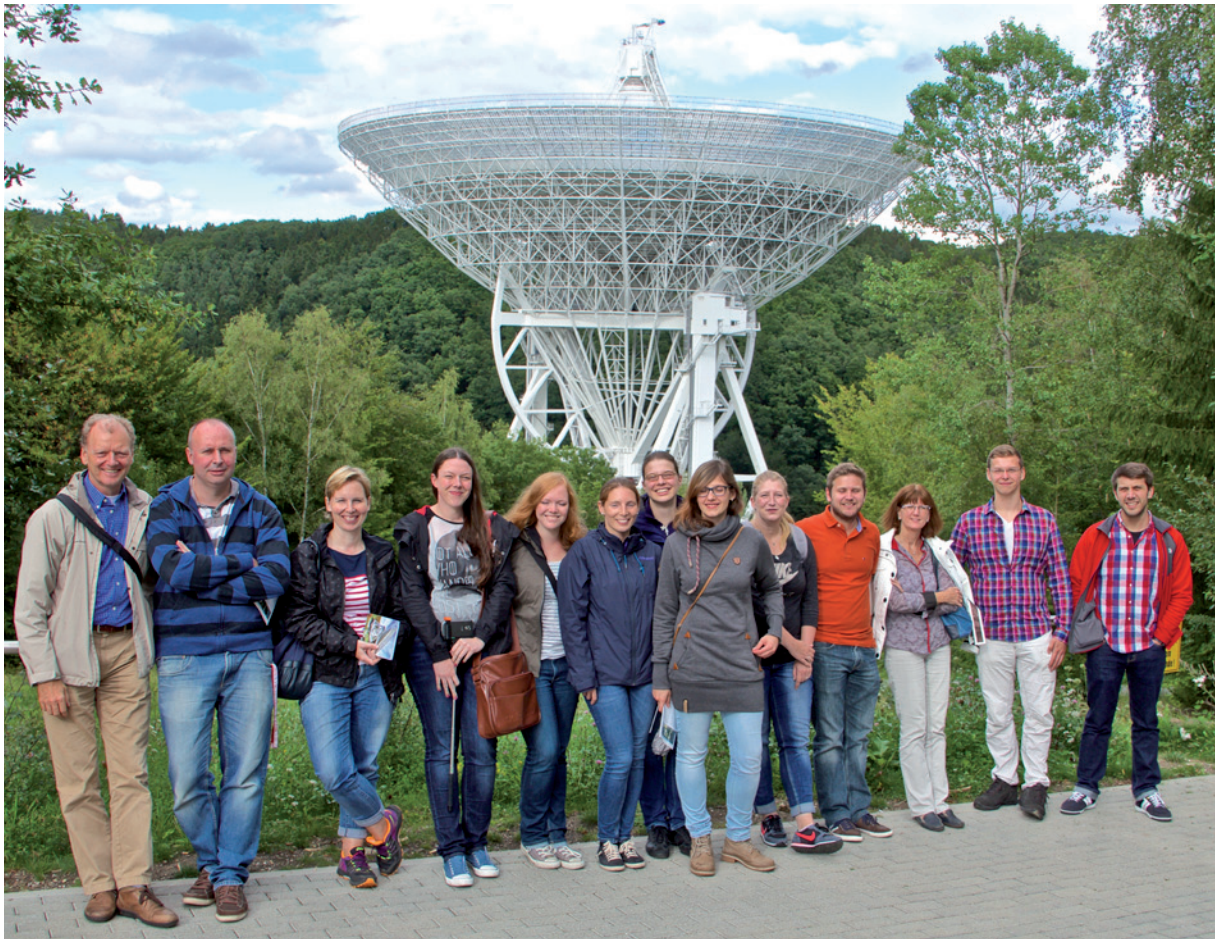
[8] Keyzer CA, De Borst MH, van den Berg E, Jahnen-Dechent W, Arampatzis S, Farese S, Bergmann IP, Floege J, Navis G, Bakker SJL, Van Goor H, Eisenberger U and Pasch A, Calcification propensity and survival among renal transplant recipients. Journal of the American Society of Nephrology : JASN, **2016**, *27*, 239-248.

[9] Neuss S, Panfil C, Duarte Campos DF, Weber M, Otten C, Reisgen U and Fischer H, Adhesion of human mesenchymal stem cells can be controlled by electron beam-microstructured titanium alloy surfaces during osteogenic differentiation. Biomedizinische Technik. Biomedical engineering, **2015**, *60*, 215-223.

[10] Stirnberg M, Maurer E, Arenz K, Babler A, Jahnen-Dechent W and Gütschow M, Cell surface serine protease matriptase-2 suppresses fetuin-A /Ahsg-mediated induction of hepcidin. Biological Chemistry, **2015**, *396*, 81-93.

[11] Vu TT, Zhou J, Leslie BA, Stafford AR, Fredenburgh JC, Ni R, Qiao S, Vaezzadeh N, Jahnen-Dechent W, Monia BP, Gross PL and Weitz JI, Arterial thrombosis is accelerated in mice deficient in histidine-rich glycoprotein. Blood, **2015**, *125*, 2712-2719.

Team



Team visiting the Radiotelescope at Effelsberg in the Eifel Mountains during our 2015 labout.