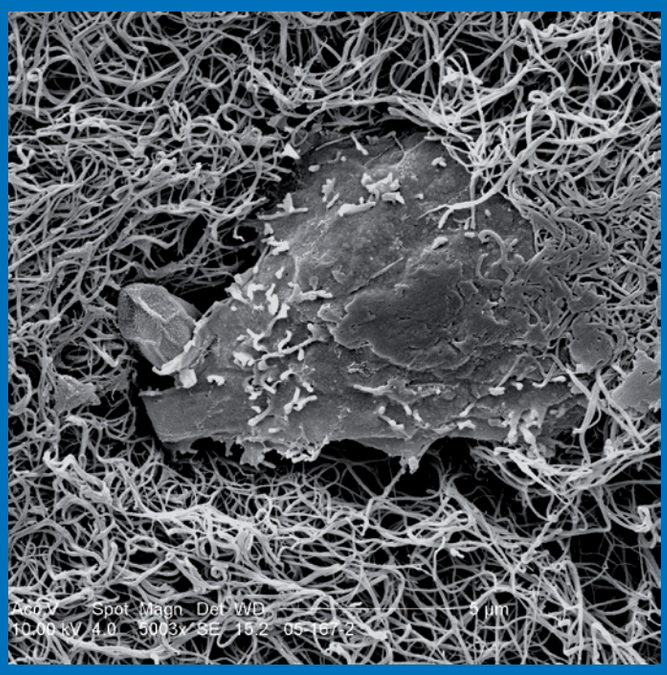
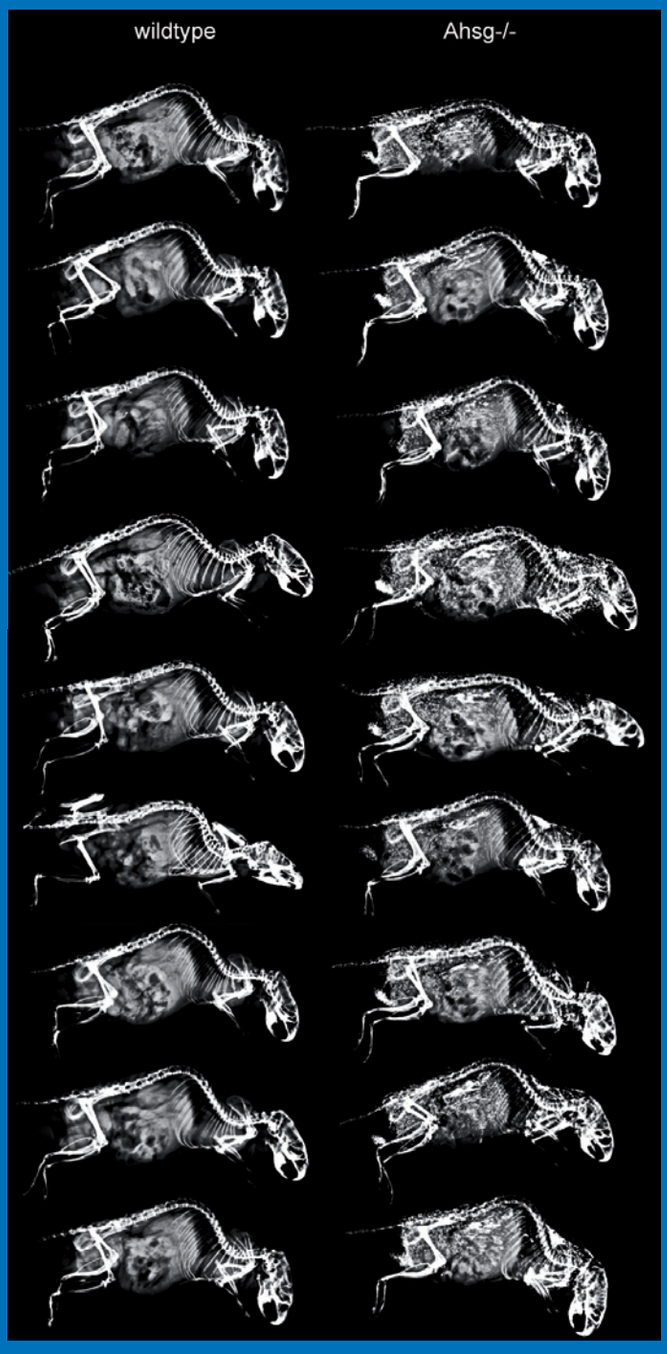
Biointerface

Faculty of Medicine

# Cell-Material Interactions: Translating Basic Science Into Clinical Applications



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***Cover Top)*** *A human mesenchymal stem cell growing in fibrin gel. A thick mesh of fibrin fibres is apparent in this scanning electron micrograph taken from a freeze-dried sample.*
***Bottom)*** *Whole body radiographs of live DBA/2 wildtype and Fetuin-A/Ahsg deficient mice. Each series represents nine mice randomly chosen from the respective strain. Note that DBA/2 wildtype mice show no overt calcification at this level of detail. In contrast, virtually all D2, Ahsg-/- mice have extensive calcification of the skin, kidneys, testes and brown fat in the neck. Heart, lung and pancreas are also heavily calcified but are blurred due to breathing and heartbeat. The fetuin-A knockout mice are thus the most calcifying mice in existence. They convincingly demonstrate that fetuin-A plays a major role in mineralization homeostasis.*

## Introduction

Last year has brought important changes in the Biointerface Group staff. Cora Schäfer and Alexander Heiss have left the group to take up industry positions. Cora and Alex, two long-term members have contributed to fetuin biology in a major way. They are first authors of some of the most highly cited papers in the field and they have trained many junior scientists, who now continue the work on fetuin biochemistry and biology. It is both sad and cheerful to see them leave. On the one hand we lose highly regarded colleagues and their valuable advice and experience, on the other side they gain jobs with a long-term perspective not readily available under the German University system. We wish both Cora and Alex the best of luck and success and we keep them as good friends.

Sabine Neuss-Stein, a former PhD student of Prof. Jahnen-Dechent and now a lecturer (Privatdozent) and independent group leader has entered a part-time appointment with the group. Sabine greatly strengthens the research topic of cell-material interaction with a view on stem cells and their application in cell-based testing and in tissue engineering. Sabine brings along a group of talented and highly motivated students who will undoubtedly broaden the scope of work and methodology in the group. Welcome!

## Mesenchymal Stem Cells in Tissue Regeneration

### (Sabine Neuss-Stein)

Human adult mesenchymal stem cells (MSC), first described by Friedenstein and coworkers in 1968, are the natural precursor cells of mesodermal tissue, such as bone, fat, muscle or cartilage. During lifetime, MSC are involved in wound healing and regeneration processes. For these processes, the stem cells have to be mobilized and recruited to the injured tissue. Since MSC reside in tissue specific stem cell niches, recruitment involves detachment of the stem cells from their niche, blood vessel diapedesis, circulation in the bloodstream, tissue transgression and finally migration to the wounded area. The underlying molecular mechanisms of this stem cell recruitment are incompletely understood.

Over the past years, we helped to clarify these mechanisms. MSC reside in their niches awaiting signals that trigger self-renewal, differentiation or stem cell recruitment. When specialized cells in the surrounding tissues need to be replaced, MSC will be recruited by cytokines and growth factors. We have previously identified hepatocyte growth factor, HGF - which is present in high concentrations in wounded tissue - as a potent chemoattractant for MSC [1]. Wounded tissues often contain clotted blood rich

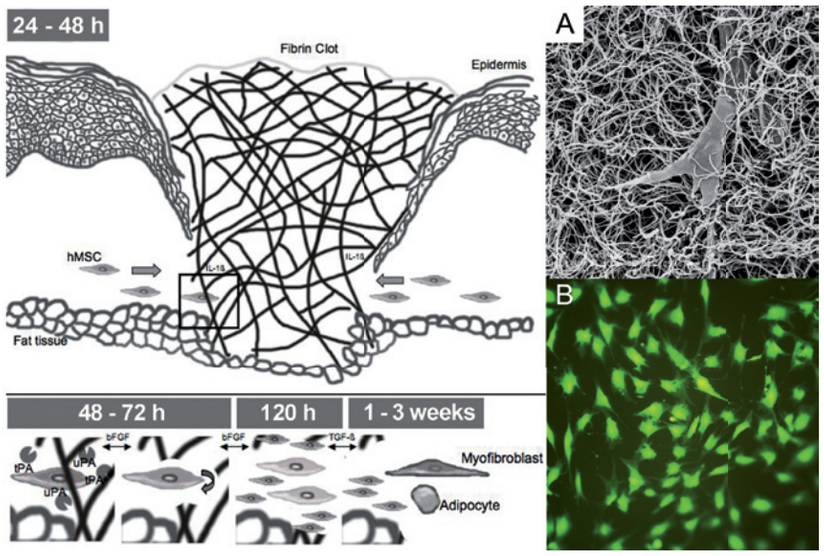



***Fig. 1: Role of mesenchymal stem cells in wound healing and tissue regeneration.*** *Schematic representation of a putative role of MSC at various phases of wound healing including fibrin degradation, cell proliferation and differentiation. A) MSC embedded in a fibrin mesh. B) Live-Dead staining demonstrating that MSC grown in fibrin are highly viable and proliferative.*

in fibrin, a "tissue glue" impeding cell invasion. We have now found that MSC secrete fibrinolytic enzymes, such as urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA) mediating fibrin degradation [2] thus allowing for fibrin clot invasion. At the tissue lesion, MSC enhance wound healing in many ways. Besides differentiation into mature cells [3, 4], MSC regulate wound healing by wound contraction [5], secretion of paracrine factors [1], and extracellular matrix remodelling [6]. These findings enable the rational design of smart biomaterial-based systems for the recruitment of endogenous MSC to chronic wounds.

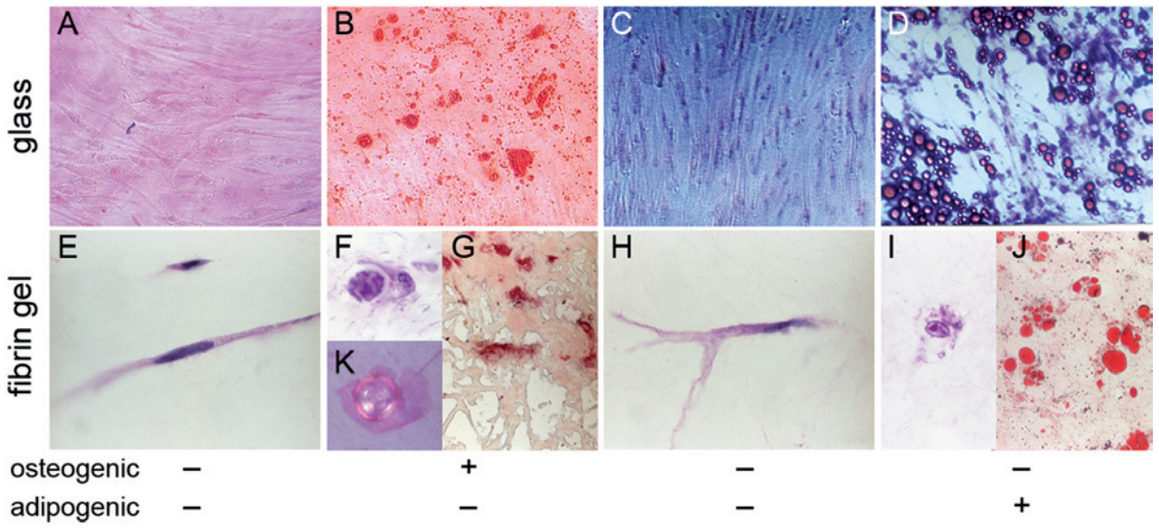
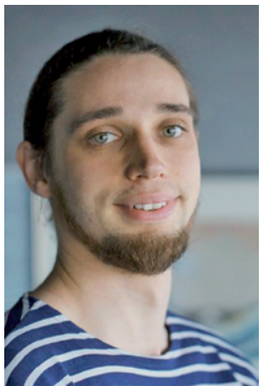



***Fig. 2: Multipotency of MSC grown as a monolayer and embedded into a fibrin clot.***
*Following 21 days of osteogenic and adipogenic induction, cells were stained with Alizarin Red (A, B) to visualize mineralized extracellular matrix of osteoblasts or with Oil Red O (C, D) to stain vacuoles of adipocytes. Differentiated cells stained strongly positive for the respective stains (B, D) while untreated cells stained negative with Alizarin Red and Oil Red O (A, C). MSC retained their multipotency when embedded into a fibrin gel. Again, after 21 days of osteogenic differentiation Alizarin Red stained positive (F, G). Following adipogenic differentiation the cells had numerous lipid vacuoles, which stained positive with Oil Red O (I, J, K).*

## Stem Cells in Biomaterials Contact

In tissue engineering, biomaterials form a blueprint for the reconstruction of damaged organs. Stem cells with their ability to differentiate into specialized, mature cell types, are promising cells for tissue engineering. In the past, the identification of suitable combinations of stem cells and biomaterials required a tedious iterative process, because single combinations had to be individually tested. Combinatorial and parallel approaches (biomaterial arrays) were introduced to allow for a faster analysis of hundreds or even thousands of biomaterials in cell contact. We developed a grid-based biomaterial test platform for the assessment of stem cell/biomaterial combinations for stem cell-based tissue engineering. Cell morphology, cell adhesion, viability, proliferation, cytotoxicity and apoptosis were analyzed in parallel using seven stem cell types (e.g. mesenchymal stem cells, hematopoietic stem cells, embryonic stem cells) and 19 natural or synthetic polymers [7]. We expanded the biomaterials testing platform by including shape memory polymers, ceramics, and semi-synthetic silk [8, 9]. On the cell side we included reprogrammed stem cells, both induced pluripotent stem cells (iPS cells) and germline-derived pluripotent stem cells (gPS cells). We also adopted the cell-based assays of cytotoxicity to a liquid handling robot [10] and we identified suitable polymers for MSC-based bone tissue engineering [3].



## The Anti-Inflammatory Role of Fetuin-A in Fetal and Newborn Life

**(Johannes Elsas)**

The plasma concentration of the liver-derived protein fetuin-A is highest in fetal life and in preterm newborns. Plasma fetuin-A is maintained at a lower level throughout childhood and adolescence [11]. We showed that fetuin-A is an important regulator of physiological and pathological calcification. In addition there is evidence that fetuin-A also strongly modulates the response of the innate immune system, decreasing pro-inflammatory cytokine release from stimulated macrophages. *In vivo* studies with rodents demonstrated that fetuin-A effectively attenuates inflammation associated with endotoxemia or sepsis: Fetuin-A-deficient mice showed a greater lethality than wildtype animals, and peripheral administration of fetuin-A dose-dependently increased survival rates. Likewise, intravenous administration of fetuin-A in a rat model of cerebral ischemia resulted in dose- and time-dependent reduction of brain infarct volume. Of the multiple mechanisms involved, interaction with the inflammation-modulating polycation spermine and prevention of an excessive release of the pro-inflammatory protein HMGB1 from injured cells seem to play a key role [12, 13].

Fetuin-A also probably has a function in fetal brain development, as numerous immunohistochemical studies have confirmed intracellularly localized fetuin-A in a transient neuronal population in fetal brains of various mammalian species, including human fetuses. The significance of these findings is underscored by the fact that fetuin-A influences both apoptosis and tumoral growth. Currently we are studying the role of intraneuronal fetuin-A in human

premature newborns with a clinical history of cerebral hypoxia-ischemia, a pathological event that is responsible for the majority of perinatally acquired cerebral injury and neurological disability. We identified neurons in various cerebral regions (including the hippocampus, the basal ganglia and the neocortex) in tissue sections from deceased premature newborns that stain strongly positive for fetuin-A. Further investigation is on its way, evaluating also the impact of the severity of the hypoxic-ischemic event, therapeutic interventions and the contribution of other inflammation-modulating substances, such as HMGB1.

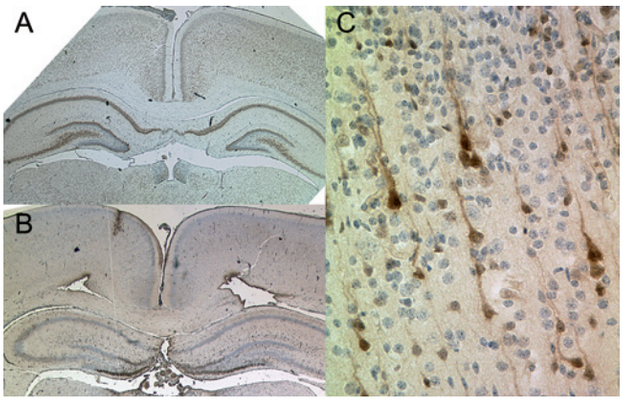



***Fig. 3: Fetuin-A distribution in the brain.*** *Neonatal rat frontal brain sections stained with A) NeuN and B) fetuin-A antibody. C) Fetuin-A positive neurons of the inner pyramidal cell layer in the neocortex of a pre-term human baby born after the 28th week of pregnancy. This picture suggests selective uptake of fetuin-A by these cells.*

## A Putative Molecular Mechanism of Calciprotein Particle Clearing

### (Marietta Herrmann)

Our previous work demonstrated an important function of fetuin-A in the formation and stabilization of calciprotein particles (CPPs). Alexander Heiss studied the physical-chemical properties of fetuin-A mineral complexes in great detail [14-17]. However, it is unclear how CPPs can be removed from the circulation *in vivo*. It is reasonable to consider fetuin-A as an opsonin aiding the clearing of the mineral particles.

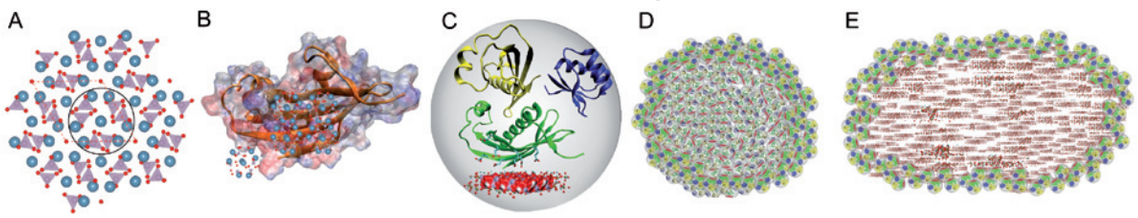
An opsonic activity of fetuin-A was first described in 1974, as van Oss and colleagues found that the presence of human serum fetuin-A enhances the phagocytosis of *E. coli* as well as *S. aureus* by human neutrophils. This finding was confirmed by other studies. For instance coating of DNA or latex particles with fetuin enhanced their uptake by mouse peritoneal and human blood monocytes. Hart and co-workers postulated a function of fetuin-A in the clearance of apoptotic cells by human macrophages; they demonstrated a dose-dependent enhancement of the uptake of apoptotic cells.

Micro- and nanoparticles have been intensively studied in relation to targeted delivery of drugs and antigens. Thiele et al. examined the effect of opsonization of different kinds of microspheres by serum albumin, immunoglobulins and fetuin-A, applied as single proteins or protein mixtures. In agreement with the earlier findings it was shown that fetuin-A enhances the uptake of most of the particles in dendritic cells, the effect was comparable with IgG opsonization. A Japanese group suggested a putative pathway responsible for the uptake of fetuin-A absorbed nanoparticles. The uptake of 50 nm polystyrene particles coated with fetuin-A by Kupffer cells could be inhibited by a known inhibitor of scavenger receptors (SRs) as well as by pre-treatment with a SR-A blocking antibody.

Further supporting the role of fetuin-A in opsonization and the immunological response is the fact that fetuin-A binds spermine. Spermine is a common biogenic amine known to inhibit the synthesis of pro-inflammatory cytokines in human peripheral blood mononuclear cells. Later on it was shown that fetuin-A is essential for the inhibition of TNF production by spermine. Thus fetuin-A acts as opsonin for the cationic molecule and enables its uptake by macrophages.

Little is known regarding a fetuin-A specific receptor, which could mediate the clearing of fetuin-opsonized particles or microorganisms. With respect to monomeric fetuin-A, controversial, but not necessarily contradictory studies described the accumulation of fetuin-A in bone, binding to lectins expressed on tumor cells and a calcium- and annexin-dependent uptake of fetuin-A in vascular smooth muscle cells. In comparison, the clearing of asialo-fetuin, the desialyated form of the glycoprotein fetuin-A, has been studied in great detail. Asialo-fetuin is taken up mainly by hepatocytes in the liver via the asialo-

***Fig. 4: Formation and stabilization of calciprotein particles.*** *A) View of the crystal structure of hydroxyapatite along the [001] axis. B) 3D view of five mineral clusters juxtaposed to the acidic β-sheet of fetuin-A domain D1. C) A computer-generated homology model structure illustrating the fetuin-A – mineral interface. D) Primary calciprotein particles (CPPs) are spherical and rather unstructured agglomerates of mineral (clusters) and fetuin-A whereas E) secondary CPPs consist of a crystalline mineral core covered by fetuin-A. In conclusion, fetuin-A effectively shields the mineral phase, leading to stable CPPs.*



glycoprotein receptor while native, fully sialylated fetuin-A escapes this route.
In conclusion a receptor specific for the clearing of fetuin-A coated particles is still missing. Currently we are studying the binding and phagocytosis of CPPs in macrophages derived from strains of mice deficient in various scavenging receptors that are candidate structures for fetuin-A binding.

## "Glowfish"
### (Yu Pan-Bartneck)

How can you tell a living animal is undergoing stress from toxic compounds? The answer is quite simple if you happen to have transgenic zebrafish expressing green flourescent protein (GFP) under the control of the heat shock protein 70 kDa promoter, Hsp70. We obtained these fish from Mary Halloran and use them for a real time survey of nanoparticle-induced toxicity.

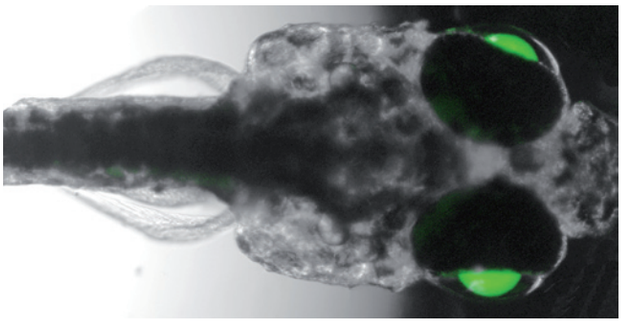


***Fig. 5: Transgenic Zebrafish Expressing Green Fluorescent Protein.*** *Freshly hatched zebrafish larva expressing GFP under the control of the Hsp70 promoter. Unchallenged, the fish express GFP in their cornea leading to a ghostly appearance in this double exposure photograph.*

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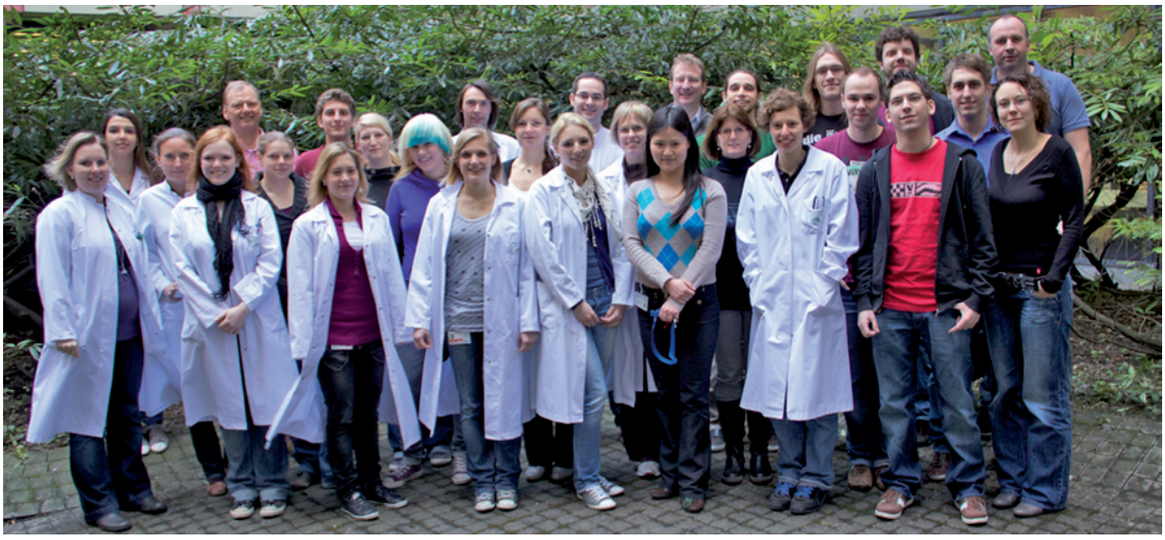

*Fig. 6: The Biointerface Group and members of the Pathology Department in February 2011.*


*Fig. 7: Biointerface meets Tut-Ench-Amun in Cologne and Yu Pan-Bartneck meets her PhD examiners at RWTH Aachen.*