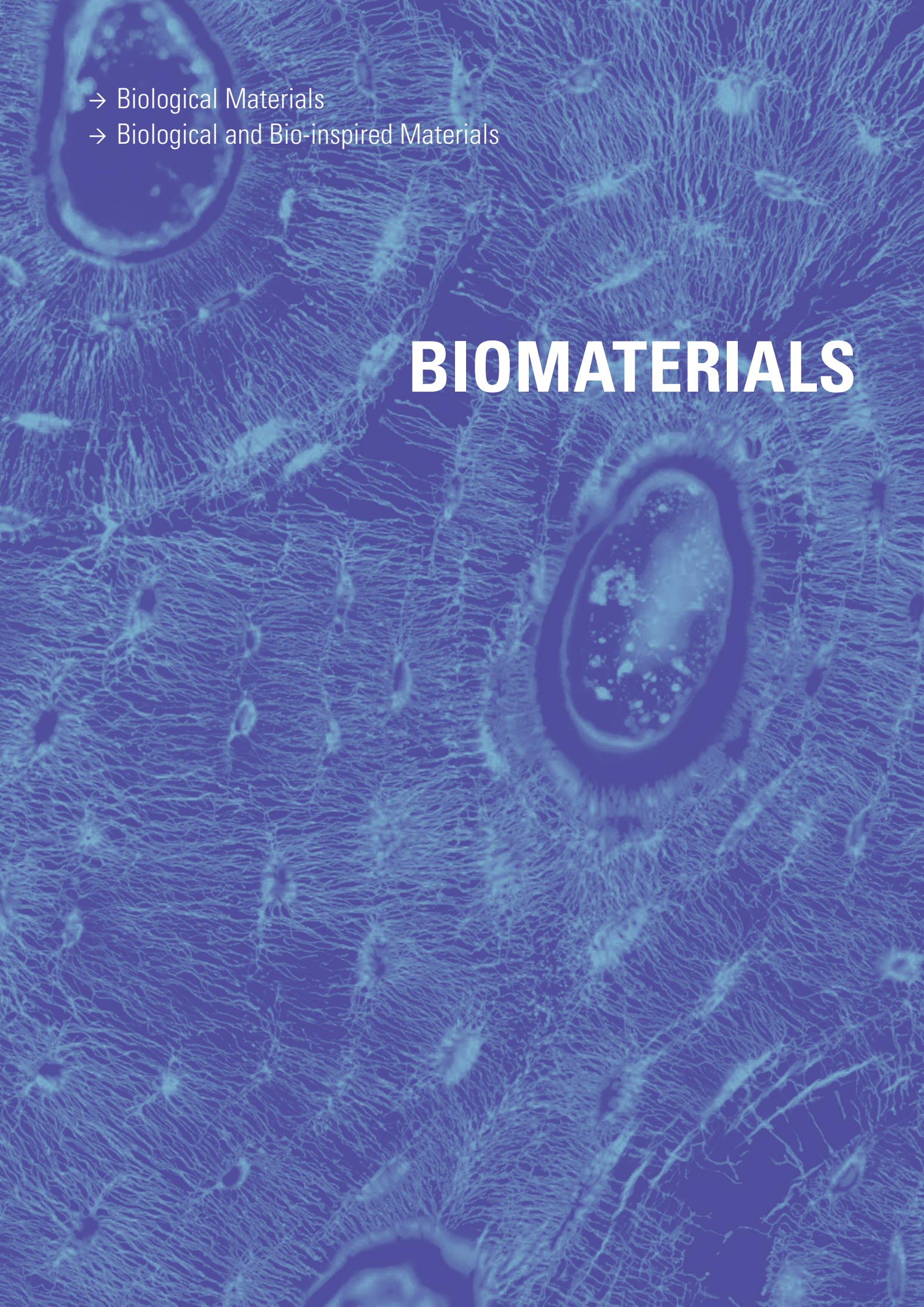
→ Biological Materials
→ Biological and Bio-inspired Materials

BIOMATERIALS



Research in the Department of Biomaterials

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Thesis: Investigation of an Al-Zn-Mg alloy using diffuse neutron scattering
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1988 and 1989: Visiting Professor (Rutgers University, New Jersey, USA)
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Since 2004: Honorary Professor of Physics at Humboldt University Berlin
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Biological Materials Science is at the center of the research program in the Department of Biomaterials. This research is inherently multidisciplinary between physics, chemistry and biology, and its principle goals are:

- To use materials science approaches for studying structure-function relationships in biological systems, with potential applications in biology or medicine;
- To study the "engineering design" which arose during the evolution of natural materials and to extract useful principles for the development of new bio-inspired materials;
- To develop new materials for contact with biological tissues, leading to implantable biomaterials or with applications in tissue engineering.

All three areas are addressed in the Department; however, there is a significantly stronger emphasis on the first two. To tackle such questions, the members of the Department have very diverse scientific backgrounds, including mathematics, physics, chemistry, materials science, geosciences, biochemistry, wood science, botany, molecular biology and dentistry.

Structure of the Department

The Department is organized into topical research groups, each of them concentrating either on a class of biomaterials (such as the plant cell wall or mineralized tissues) or on a special methodology (such as synchrotron research or mathematical modeling). In this way, a strong expertise in a given field is maintained in each of the groups and important scientific problems at the interface between these disciplines are addressed by interaction and collaboration between them. Typically, the research groups encompass – in addition to the group leader – several doctoral students, postdocs, one or two technicians, as well as the responsibility for laboratories and the larger instrumentation of the institute. In addition to the research groups, several independent researchers, some of them with individual grants (e.g. from the Humboldt Foundation) work on chosen scientific projects, sometimes mentoring a student, but without responsibility for a larger group.

Methodological Approaches

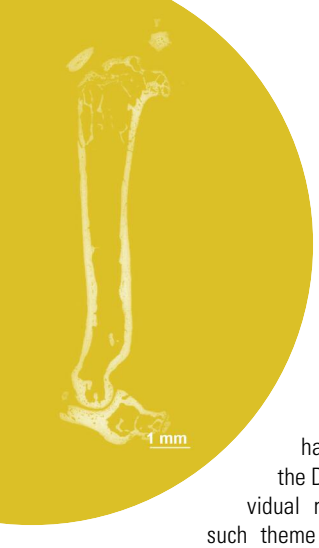
Generally, the experimental approach is based on multi-method imaging where different probes are used to image the same specimen. This provides information on different features of the materials such as micro-structure, chemical composition, or mechanical properties in a position-resolved manner with micron-range resolution . We are currently using x-ray tomography; scanning electron microscopy and scanning x-ray diffraction to characterize micro- and nanostructure (see, e.g. reports by *W. Wagermaier* and *P. Zaslansky*). We have established polarized and confocal Raman imaging to provide information on chemical composition and fiber orientation (see report by *A. Masic*), and we use nano-indentation as well as acoustic microscopy to estimate local mechanical properties. The strength of this multi-method approach is that the different parameters measured on the same specimen can be correlated at the local level with micron-scale spatial resolution. This facilitates the extraction of structure-property relationships even in extremely heterogeneous materials with hierarchical structure. Additionally, we are currently developing the infrastructure required to probe these materials at the biochemical level in order to better understand how specific molecular-level features of the biopolymeric building blocks influence bulk material properties (see report by *M. Harrington*).

In a second type of approach, we study *in situ* changes in various materials (e.g. due to mechanical stress or to chemical or thermal processing) by time-resolved scattering or spectroscopy during mechanical deformation or thermal or hygroscopic treatment. This gives insight into the molecular and supramolecular mechanisms which are responsible for the noteworthy properties of these materials. In some cases, such measurements can be performed in the laboratory (e.g. with Raman or infrared spectroscopy or in the environmental scanning electron microscope), but in many cases synchrotron radiation is needed (e. g. for x-ray diffraction or small-angle scattering). A dedicated beamline end station for scanning small- and wide-angle scattering and fluorescence spectroscopy is operated at the synchrotron BESSY at the Helmholtz Zentrum Berlin (see report by *B. Aichmayer*).

These efforts are complemented by a significant effort in mathematical modeling, which is always closely tied to the experimental work in the department. Typically, modeling and experimentation go hand in hand with the research projects (see for example the reports by *J.W.C. Dunlop* and *R. Weinkamer*).

Visiting Scholars

Several experienced scientists have been spending significant time in the Department. *Franz Dieter Fischer*, professor of mechanics at the Montanuniversität Leoben (Austria) recipient of the Alexander von Humboldt Award, came for many short visits, which helped advance the mathematical modeling of tissue growth in particular (see report by *J.W.C. Dunlop*). *Hartmut Metzger* arrived in the beginning of 2010 from the European Synchrotron Radiation Facilities (ESRF), where he had been a staff scientist and group head responsible for several beamlines. He brought many years of experience in x-ray diffraction, in particular with grazing incidence and using coherent beams, to our Department. *Emil Zolotoyabko*, professor of materials science at the Technion (Israel Institute of Technology) spent several months of a sabbatical in the Department. He offered his general knowledge of materials science in many discussions, as well as an advanced course in crystallography, which was well attended by the scientists in the Department. In addition to developing new collaborations, our visiting scholars play an important role in the mentoring of young scientists, and we are most grateful to them for this very important contribution. Recently, it was announced that *Yves Bréchet*, professor of materials science at the Institut National Polytechnique de Grenoble (INPG) and at the Institut Universitaire de France (IUF) will receive the Gay Lussac-Humboldt Award to visit our Department in 2011.



Bone Research

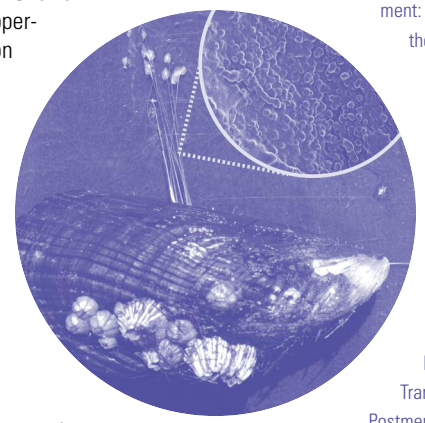
The director and the group leaders have defined overarching themes for the Department where many of the individual research groups collaborate. One such theme is bone research. The rationale behind these studies is that osteoporotic bone fractures, which have generally been associated with bone loss, may also be linked to changes (age- or disease-related) in the bone material itself. In collaboration with the Ludwig Boltzmann Institute of Osteology (Vienna, Austria), we study the changes in bone material quality in osteoporotic bone before and after treatment with various strategies. This is one area where we collaborate with industry, mostly in the framework of large clinical studies. Publications of the last two years addressed the effect on osteoporotic bone material following treatment with strontium ranelate. In particular, we showed that Sr is not only acting on the bone cells but gets incorporated into the mineral particles during treatment **[1, 2]**. Long-term treatment with the bisphosphonate alendronate, however, does not seem to affect the structure of the bone material significantly **[3]**. The adaptation of bone structure by the collaborative action of bone-forming and resorbing cells is modeled by the group of Richard Weinkamer. The importance of osteocytes, which are cells embedded in the bone matrix is studied within the framework of a BMBF consortium by the group of *Wolfgang Wagermaier* (see their reports and the references therein). Finally, the Department is very active in a consortium with the Charité Hospital Berlin (Julius Wolff Institute) and the Berlin-Brandenburg School of Regenerative Therapies, a graduate school financed by the German Science Foundation (DFG) through the excellence initiative. In this consortium, we study bone regeneration by characterizing and modeling the healing process, (see the reports by *Inderchand Manjubala* and by *Richard Weinkamer*) and explore routes towards bio-inspired tissue-engineering scaffolds **[4]** (see also report by *John W.C. Dunlop*).

Bio-Inspired Actuating Materials

Humidity-driven actuation plays an important role in plant movement, in seed dispersal and in the generation of growth stresses in trees **[5]**. We are studying natural systems where the material deformation is triggered by humidity as an external stimulus. The group of *Ingo Burgert* dedicates a large effort to this subject and additionally, we maintain a collaboration with *Rivka Elbaum*, a former postdoc in the Department and now at the Hebrew University (Israel). Numerical modeling of complex movements is addressed in the report by *John Dunlop*, with particular emphasis on the influence of the internal structure on deformation patterns. The report by *Matthew Harrington* shows the specific example of a desert plant seed capsule that opens upon contact with water droplets, despite consisting of non-living material. Recently, we started a more concentrated activity to investigate the interaction of water with deformable materials, in which we focus on wood fibres (see report by *Ingo Burgert*), as well as model material systems **[6]**. A new independent researcher, *Luca Bertinetti*, is starting to concentrate on these aspects.

Biomineralization

Two groups are focusing on issues related to biomineralization. *Damien Faivre*, who was just awarded an ERC Starting Grant from the European Research Council, works on elucidating how bacteria control the growth of magnetite nanoparticles through the interaction with specialized proteins. His group also addresses bio-inspired engineering problems in the context of nanorobotics and medical imaging with magnetic nanoparticles (see his report). *Barbara Aichmayer* leads a group working both on natural mineralized tissues (such as crayfish teeth) and on bio-inspired hybrid materials, based on polymers and mineral (see her report). She is also responsible for the operation of our synchrotron beamline MµSpot at the BESSY synchrotron of the Helmholtz Center Berlin. Together with the visiting scholar *Dieter Fischer*, we also model various aspects of biomineralization **[7]**.



Load-Bearing Natural Materials

Matthew Harrington is currently building a group focused on understanding the biochemical strategies utilized in load-bearing natural materials. In his report he describes the role of reversible metal coordination cross-links in the self-healing byssus fibres, which mussels use to attach to rocks. An essential component of these self-healing materials are sacrificial bonds, which have also been investigated theoretically **[8]**. In addition, several independent researchers report on their work on calcified cartilage in shark skeletons (*Mason Dean*), on structure–function relationships in the spider cuticle (*Yael Politi*), on Raman imaging of biological tissues, in particular collagen (*Admir Masic*) and on human teeth and dental restorations (*Paul Zaslansky*). Two of these independent researchers are financed by their own Humboldt Fellowships (*Dean* and *Politi*), and the remainder are supported by a Max Planck Research Prize, awarded to *Peter Fratzl* in 2008. The Gottfried Wilhelm Leibniz Prize 2010 will allow further increasing this research activity.

The majority of the research in the Department of Biomaterials involves collaborations – within the Department, with other Departments in the Institute and with many outside partners around the world who all deserve our sincere gratitude for cultivating and fostering such positive and constructive partnerships.

Peter Fratzl
Director of the Department of Biomaterials

References:

[1] Li, C., Paris, O., Roschger, P., Paschalis, E.P., Klaushofer, K., Fratzl, P.: Strontium is incorporated into mineral crystals only in newly formed bone during strontium ranelate treatment. J. Bone Miner. Res. **25**, 968-975 (2010)

[2] Roschger, P., Lombardi, A., Misof, B.M., Maier, G., Fratzl-Zelman, N., Fratzl, P., Klaushofer, K.: Mineralization Density Distribution of Postmenopausal Osteoporotic Bone is Restored to Normal After Long-Term Alendronate Treatment: qBEI and sSAXS Data From the Fracture Intervention Trial Long-Term Extension (FLEX). J. Bone Miner. Res. **25**, 48-55 (2010)

[3] Roschger, P., Manjubala, I., Zoeger, N., Meirer, F., Simon, R., Li, C., Fratzl-Zelman, N., Misof, B.M., Paschalis, E.P., Streli, C., Fratzl, P., Klaushofer, K.: Bone Material Quality in Transiliac Bone Biopsies of Postmenopausal Osteoporotic Women After 3 Years Strontium Ranelate Treatment. J. Bone Miner. Res. **25**, 891-900 (2010)

[4] Willie, B.M., Petersen, A., Schmidt-Bleek, K., Cipitria, A., Mehta, M., Strube, P., Lienau, J., Wildemann, B., Fratzl, P., Duda G.: Designing biomimetic scaffolds for bone regeneration: why aim for a copy of mature tissue properties if nature uses a different approach? Soft Matter **6**, 4976-4987 (2010)

[5] Fratzl, P., Barth, F.G.: Biomaterials systems for mechanosensing and actuation. Nature **462**, 442 - 448 (2009)

[6] Prass, J., Müter, D., Fratzl, P., Paris, O.: Capillary-driven deformation of ordered nanoporous silica. Appl. Phys. Lett. **95**, 083121/1-3 (2009)

[7] Fratzl, P., Fischer, F.D., Svoboda, J., Aizenberg, J.: A kinetic model of the transformation of a micropatterned amorphous precursor into a porous single crystal. Acta Biomater. **6**, 1001-1005 (2010)

[8] Hartmann, M.A., Fratzl, P.: Sacrificial ionic bonds need to be randomly distributed to provide shear deformability. Nano Lett. **9**, 3603-3607 (2009)

Bone Regeneration

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Regeneration of bone is a complex process involving both formation and resorption process. While bone is known to grow and heal itself, in some critical condition beyond the limit the bone formation is regulated by biochemical, mechanical and cellular mechanism. We focus on understanding the development of new bone formation under natural growth, fractured conditions and mimicked in-vitro conditions.

Tissue Formation under *in-vitro* Conditions by Osteoblast Cells

An *in-vitro* system in which the behaviour of tissue formation by the osteoblast cells (MC3T3-E1) in defined geometries can be monitored in real time was established with a standard hydroxyapatite material. Since material properties of scaffolds like surface topography, stiffness and geometry play a vital role in cell survival and their responses, linear copolymer polyether urethanes with different stiffness were used as three dimensional scaffolds. These scaffolds were provided by our SFB 760 project partner, Andreas Lendlein, GKSS Institute for Polymer Research in Teltow. It was observed that the cells do respond differently in different seeding conditions, depicting different delay times of tissue formation on these polymer scaffolds (**Fig 1a**) and more interestingly the tissue formation kinetics showed a two stage behavior wherein the late stage was similar to that of hydroxyapatite scaffolds (**Fig 1b**) **[1]**.

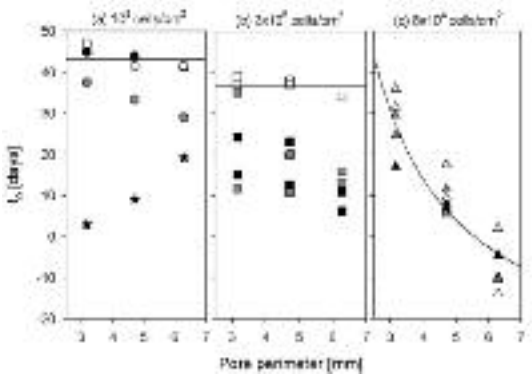

Fig. 1a: Delay time (t0) plotted as a function of pore size (perimeter). White, grey and black points represents polymer with different stiffness and star represents standard hydroxyapatite scaffold.

Apart from stiffness, the cells do respond to growth factors such bone morphogenetic proteins, BMP-2. But the responses of the BMP-2 on tissue formation are still not known and this task was carried out in cooperation with Petra Knaus, Freie University, Berlin. The continuous BMP-2 application increased proliferation and differentiation of pre-osteoblastic MC3T3-E1 cells. These observations made are of direct use in the optimization of scaffold design and has been the focus of a doctoral work **[2]**.

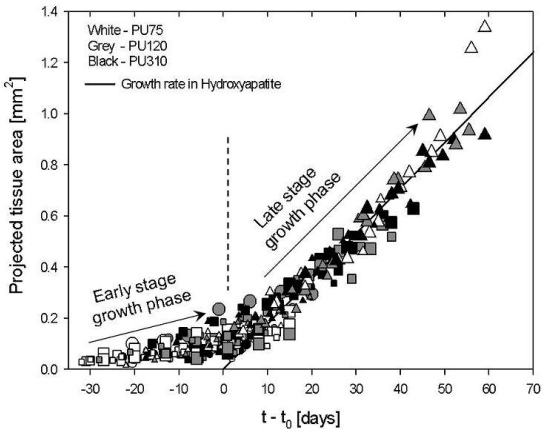

Fig. 1b: Normalized PTA data showing two stages of tissue growth in polymeric scaffolds.

Bone Healing and Regeneration

When bone is injured, a callus is formed enveloping the fracture site and is eventually remodeled back into bone to fully restore the initial morphology and function of the skeleton. While the histological evaluations describe the spatial and temporal distribution of the various tissue types comprising the callus, it is of vital importance to evaluate and understand local variations in callus material properties at the micro- and nano-scale. The investigation of the spatial distribution and temporal sequence of ultra-structural and mechanical properties of callus tissues over the course of healing has been the focus of a doctoral work **[3]**. This project is in cooperation with Georg Duda and colleagues, at Julius Wolff Institute, Charité-Universitätsmedizin Berlin where the bone healing experiments is carried out both in small and large animal models.

Measurements on similar regions of the same samples, with nanoindentation, scanning small angle X-ray scattering (SAXS) together with environmental scanning electron microscopy (ESEM) revealed the heterogeneous local mechanical property, size and orientation of bone mineral particles within the regenerating callus tissues **[4, 5]**. Both studies showed that the callus formation is characterized by two waves of bone formation. Starting from the periosteal region, a structurally disordered and mechanically inferior woven tissue was deposited first and replaced later by more

lamellar-like tissues with thicker and more aligned mineral particles, lower mineralization heterogeneity and higher mechanical competence (**Fig 2**).

In light of such findings, the X-ray study was extended from laboratory resolution to synchrotron resolution and from 2D to 3D. The simple but effective representation of 3D SAXS data and the reconstruction of 3D SAXS patterns enable a direct visualization of mineral alignment in the investigated sample volume, which provides insight into the 3D structural properties of the callus material as well as their relation with its mechanical performance **[6]**.

Bone regeneration and remodelling in other cases such as treatment with a stainless steel implant **[7]** or with a drug for osteoporosis **[8]** was also studied.

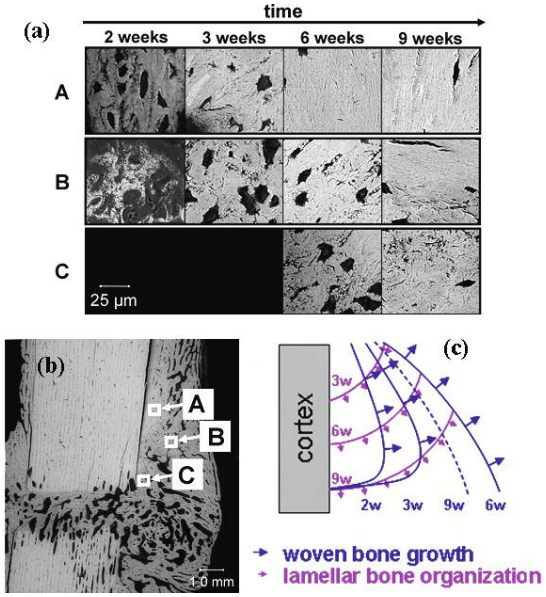

Fig. 2: (a) Back-scattered electron images (4000×) at three different locations, A, B and C with the callus as shown in the overview image (b). Different tissue types are visible: (i) an unmineralized fibrous tissue as observed in C at 2 and 3 weeks, (ii) a mineralized but poorly organized woven tissue as observed in A and B at 2 weeks and (iii) mostly lamellar bone as observed in A at 9 weeks. (c) It is proposed that the transformation from structure (i) to (ii) and from (ii) to (iii) occurs in two sequential "waves" propagating along the cortex towards the osteotomy gap.

Bone Growth and Development

The understanding of the mineralization process in vertebrates is of substantial interest since less is known about how mineral crystals nucleate, grow and organize themselves from the beginning of bone development and the question of the first-formed mineral phase is still controversially discussed. This study is a part of BSRT graduate school in Berlin, in cooperation with Stefan Mundlos, MPIMG. Using scanning small angle X-ray scattering technique at lab source as well as using microbeam line at BESSY, several remarkable results were obtained such as strong differences in shape and arrangement of the mineral particles between fetal and postnatal bone, indicating two different types of bone tissue. (i) Fetal bone tissue is characterized by spotty mineralization, short but relatively thick mineral crystals and no preferred orientation of the minerals, and (ii) the postnatal bone tissue can be described by continuous mineralization, long and slender crystals where length and thickness increase simultaneously with age and highly oriented minerals. This further confirms that possibly there is a strong change in tissue organization at birth and maybe not all calcium is bound in crystalline HA in fetal bone, possibly indicating the formation of a precursor phase of HA during early bone development.

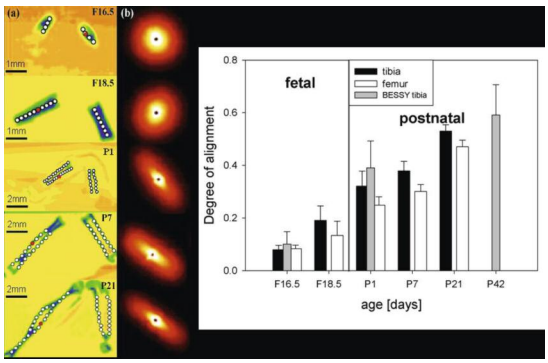

Fig. 3: (a) Radiography to define measuring positions of the SAXS measurements (b) representative two dimensional SAXS pattern of a single measurement point and (c) Rho-parameter, a measure of the degree of mineral particle alignment, analyzed dependent on age.

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References:

[1] Kommareddy ,K.P., Lange, C., Rumpler, M., Dunlop, J.W.C., Manjubala, I., Cui, J., Kratz, K., Lendlein, A., Fratzl, P. Two-stages in three-dimensional in-vitro growth of tissue generated by osteoblast-like cells. Biointerphases **5**, 45 (2010).

[2] Kommareddy, K.P. Regulation of the biosynthesis of extracellular matrix tissue by biomaterial scaffolds of different Stiffness, geometry and growth factors Doctoral thesis, Freie University Berlin (2010).

[3] Liu, Y. The micro-mechanical and structural properties of callus tissue during bone healing, Doctoral thesis, Humboldt University (2010).

[4] Manjubala, I., Liu, Y., Epari, D.R., Roschger, P., Schell, H., Fratzl, P., Duda, G.N. Spatial and temporal variations of mechanical properties and mineral content of the eternal callus during bone healing. Bone **45**, 185 (2009).

[5] Liu, Y., Manjubala, I., Schell, H., Epari, D.R., Roschger, P., Duda, G.N., Fratzl, P. Size and habit of mineral particles in bone and mineralized callus during bone healing in sheep. J. Bone Miner. Res. **25**, 2029 (2010).

[6] Liu, Y., Manjubala, I., Roschger, P., Schell, H., Duda, G.N., Fratzl, P. Mineral crystal alignment in mineralized fracture callus determined by 3D small-angle X-ray scattering J. Phys.:Conf. Ser. **247** 012031 (2010).

[7] Ballarre, J., Manjubala, I., Schreiner, W.H., Orellano, J.C., Fratzl, P., Ceré, S. Improving the osteointegration and bone-implant interface by incorporation of bioactive particles in sol-gel coatings of stainless steel implants. Acta Biomaterialia **6**, 1601 (2010).

[8] Roschger, P., Manjubala, I., Zoeger N, Meirer, F., Simon, R., Li, C., Fratzl-Zelman, N., Misof, B.M., Paschalis, E., Streli, C., Fratzl, P., Klaushofer, K. Bone material quality in transiliac bone biopsies of postmenopausal osteoporotic women after 3 years strontium ranelate treatment. J. Bone Miner. Res. **25**, 891 - 900 (2010).

Mechanobiology

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The structure of bone and its constituent material is constantly changing due to processes executed by different specialized cells. The stiffness of the bone material is changed by incorporation of mineral, the bone material is continuously remodeled and, after fracture, bone is able to heal. In these processes the behavior of cells is partly controlled by mechanical stimuli. How mechanical stimulation regulates in detail cell behavior is very challenging to study in animal experiments. Computer experiments, however, are an alternative approach to test our understanding of the mechanobiological control of processes in bone. In a computer model different hypotheses of cell reaction can be implemented, and the resulting consequences on the bone structure can then be compared with experiments.

Bone Healing

During bone healing a callus is temporarily formed around the fracture site for mechanical stabilization. Stem cells migrating to the fracture site are the biological basis for the formation of different tissues within this callus. The differentiation of the stem cells into specialized, tissue forming cells is partially mechanically controlled. In this way, low mechanical stimulation allows direct formation of bone, whereas strong stimulation results in cartilage formation. We studied the mechanobiological control of bone healing based on animal experiments on sheep with a histological documentation of the time course of the healing process performed at the Julius Wolff Institute at Charité, Berlin. The first challenge was to condense the available histological data in a succession of images that reflect the normal course of healing in sheep **[1]**. The inter-individual differences between sheep required the development of special image averaging tools to conserve the information of the temporal and spatial arrangement of different tissues in the fracture callus. Not only the arrangement of tissues evolve with time, but also the mechanical properties of the tissues themselves leading to a strong mechanical heterogeneity in the newly formed bone. The incorporation of recent experimental data in a Finite Element model allowed us to calculate the effect of this heterogeneity on the local strains within the callus **[2]**. With a phenomenological computer model of the healing process, we then investigated how the choice of the threshold values of the mechanical stimulus for bone and cartilage formation influence the course of healing (**Fig. 1**). It turned out that good agreement with the experimentally observed presence of cartilage in the callus is obtained only within a very restricted range of the threshold values and when assuming the outer skin of the broken bone to be the main source of the stem cells (**Fig. 2**) **[3]**.

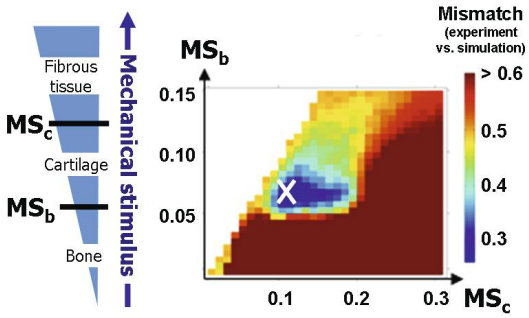

Fig. 1: Left, mechanical control of tissue formation during bone healing. Low stimulation leads to direct bone formation, whereas a higher stimulus results first in cartilage formation. Right, phase diagram summarizing a parameter study, where the threshold values of the mechanical stimulus for bone formation (MS_b) and cartilage formation (MS_c) were varied. Colors denote the agreement between experimental and simulational images with the white cross corresponding to the minimal mismatch.

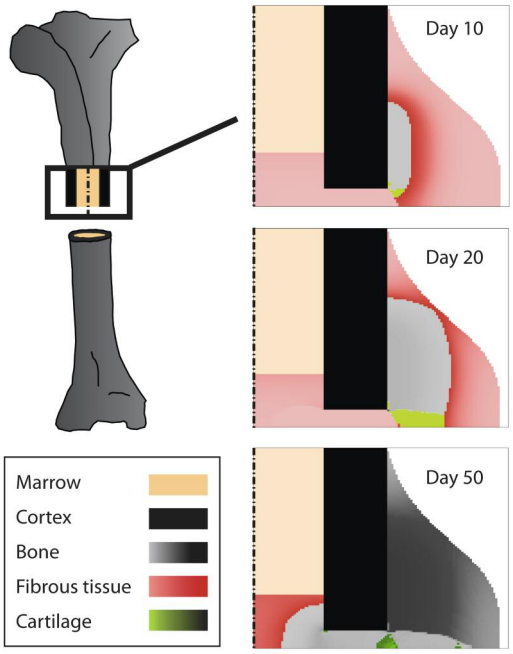

Fig. 2: The course of healing in good agreement with experimental observations obtained by a mechanobiological computer model. In the simulational images only the upper right part of the fracture with the broken cortex in black is shown. At intermediate stages of healing the bridging of the two cortex ends occurs via cartilage outside of the fracture gap.

Bone Remodeling and Structural Adaptation

The network-like architecture of trabecular bone is continuously remodeled by resorption and deposition of small bone packets from the bone surface. This remodeling process allows the trabecular structure to adapt itself to mechanical needs **[4]**. Striking evidence for the structural adaptation of

trabecular bone architecture can be found in the anisotropic arrangement of trabeculae in the proximal femur of humans and primates (**Fig. 3**). In cooperation with anthropologists, we made use of the natural variation of loading caused by a different main locomotor behavior of different primates and quantified the heterogeneity and anisotropy of the bone architecture **[5]**. Comparing the structure within the femoral head between a primate, which predominately walks, to one, which uses mainly the arms for locomotion, showed stronger anisotropy of the bone under higher load (**Fig. 3**).

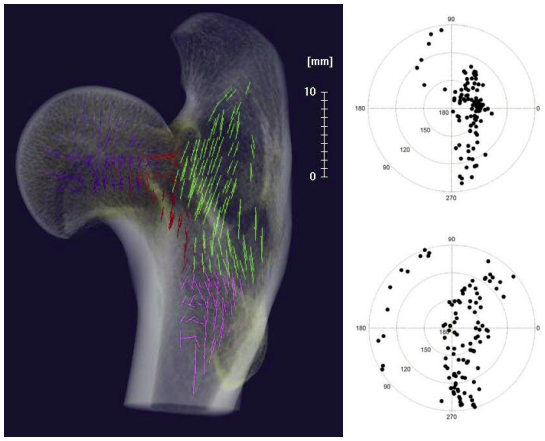

Fig. 3: Proximal femur of a baboon. The straight lines denote the local anisotropy of the trabecular bone architecture. Orientation of the lines represent the local main direction of the trabeculae, their lengths are proportional to the local degree of anisotropy. Different colors denote different anatomical regions: femoral head (blue), neck (red), shaft (pink) and greater trochanter (green). Right, the projection of the main local directions of the trabeculae onto a lower hemisphere, shows that the architecture for the "walking" baboon (top) is more anisotropic than the "brachiating" gibbon (bottom).

On the cellular level, structural adaptation to mechanical needs is realized via a mechanical control of the remodeling process. Osteocytes embedded within the bone matrix are thought to act as mechanical sensors, which signal to the bone surface the mechanical need for bone resorption or deposition. Using computer simulations we studied the influence of specific components in the mechanobiological system of cell interaction. First, different hypotheses were tested of how the mechanical stimulus for bone remodeling is integrated by osteocytes. A collective (summed) signal from multiple osteocytes as opposed to an individual (maximal) signal from a single osteocyte was found to lead to lower inner porosity and surface roughness of the simulated bone structure **[6]**. This observation can be interpreted that collective osteocyte signaling provides an effective surface tension to the remodeling process. Second, the relation between the mechanical signal reaching the cells at the bone surface and the probability for local bone resorption or deposition was studied. Simulations indicate that a threshold value for the mechanical stimulus has to be overcome to strongly activate deposition **[7]**.

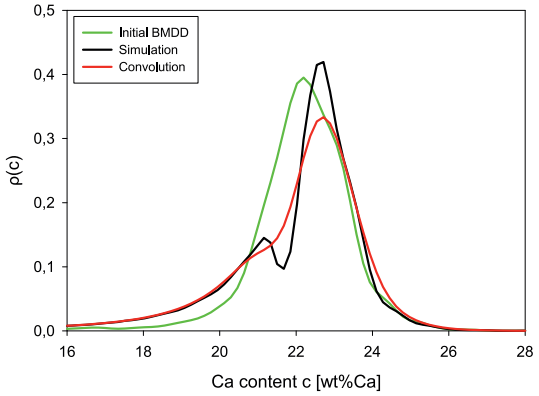

Fig. 4: Simulated time evolution of the bone mineralization density distribution (BMDD) after treatment with parathyroid hormone. The initial configuration (green) was obtained by deconvolving the measured BMDD before treatment. After 1.5 years the simulated BMDD (black) shows the formation of a second peak at about 21 wt% Ca. A measurement with a standard acquisition time of 100 seconds would show only a shoulder, but not a second peak (red).

Bone Mineralization

The continuous incorporation of mineral in a newly formed bone packet together with bone remodeling gives rise to a patchwork structure of bone packets with different mineral content. This material heterogeneity of bone can be quantified in a frequency distribution called the bone mineralization density distribution (BMDD). The BMDD has proven to be a sensitive diagnostic tool for bone diseases. Mathematical modeling allows connecting pathological changes in the BMDD with disturbances in bone remodeling and mineralization.

One limitation of the comparison between theoretical prediction and BMDD measurements is due to the fact that the experimental data are affected by the stochastic nature of the backscattering of electrons and the finite acquisition time. We have devised an approach using mathematical tools of regularization to deconvolve and correct measured BMDDs. As a result, the reference BMDD for healthy human adults could be defined with improved precision. This correction together with our computer model can further help to recover multiple peaks in the BMDD, which were smeared over by the measurement (**Fig. 4**). Our mathematical model was also applied to situations where the mineralization process is disturbed. This can occur, for example, by a tumor, which deranges mineral homeostasis by secreting hormones **[8]**. The inadequate mineralization in osteomalacia and the increased bone turnover at menopause both lead to a shift of the BMDD histogram towards lower mineral contents, in comparison to a healthy reference. With the use of mathematical modeling, it became possible to differentiate the time evolution of the BMDD, for both disease scenarios.

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References:

[1] A. Vetter, D.R. Epari, R. Seidel, H. Schell, P. Fratzl, G.N. Duda, R. Weinkamer: Temporal tissue patterns in bone healing of sheep. J. Orthopaed. Res. **28**, 1440 (2010).
[2] A. Vetter, Y. Liu, F. Witt, I. Manjubala, O. Sander, D.R. Epari, P. Fratzl, G.N. Duda, R. Weinkamer: The mechanical heterogeneity of the hard callus influences local tissue strains during bone healing: a Finite Element study based on sheep experiments. J. Biomech., **44**, 517 (2011)
[3] A. Vetter, F. Witt, O. Sander, G.N. Duda and R. Weinkamer: The spatiotemporal arrangement of different tissues during bone healing as a result of simple mechanobiological rules. Biomech. Model. Mechan., in press.
[4] R. Weinkamer and P. Fratzl: Mechanical adaptation of biological materials – the examples of bone and wood. Mat. Sci. Eng. C, in press.
[5] P. Saparin, H. Scherf, J.-J. Hublin, P. Fratzl, R. Weinkamer: Structural adaptation of trabecular bone revealed by position resolved analysis of proximal femora of different primates. Anat. Rec. **294**, 55 (2011).
[6] M.A. Hartmann, J.W.C. Dunlop, Y.J.M. Bréchet, P. Fratzl, P. and R. Weinkamer: Trabecular bone remodeling simulated by a stochastic exchange of discrete bone packets from the surface. J. Mech. Behav. Biomed., in press.
[7] J.W.C. Dunlop, M.A. Hartmann, Y.J. Bréchet, P. Fratzl and R. Weinkamer: New Suggestions for the Mechanical Control of Bone Remodeling. Calcif. Tissue Int. **85**, 45-54 (2009).
[8] K. Nawrot-Wawrzyniak, F. Varga, A. Nader, P. Roschger, S. Sieghart, E. Zwettler, K.M. Roetzer, S. Lang, R. Weinkamer, K. Klaushofer, N. Fratzl-Zelman: Effects of tumor induced osteomalacia (TIO) on the bone mineralization process. Calcif. Tissue Int. **84**, 313-323 (2009).

Biochemical Strategies in Load-Bearing Natural Materials

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Biological organisms synthesize a diverse assortment of robust load-bearing materials from various combinations of simple organic biopolymers such as proteins or sugar chains and inorganic components such as ions or minerals. Additionally, biological materials have evolved to do this with a highly economical use of resources under environmentally friendly processing conditions. The resulting biologically produced materials often have interesting and technologically attractive properties that are not yet realized in engineering materials. For these reasons, it is of interest to understand the structure-function relationships that underlie the performance of biological materials.

Fig. 1: Mussels secure themselves to available surfaces with an array of fibers known as the byssus. Byssal threads are an attractive model system for biomimetic investigation because of their interesting mechanical properties, especially the ability to self-repair following load-induced damage.

In the new group "Biochemical strategies in load-bearing natural materials" (formed July 2010), we utilize a variety of methods including those in biochemistry, molecular biology, materials science, spectroscopy, and polymer science with the aim of establishing basic connections between the biochemical components of natural materials, their hierarchical organization and the resulting material properties. One model system that provides a unique opportunity for probing such questions is the byssus of marine mussels (**Fig. 1**). Mussels use the biopolymeric attachment fibers that make up the byssus to create a secure holdfast on surfaces in rocky seashore environments. The mechanical properties of byssal threads are specially tailored for their role as an abrasion resistant shock absorber and additionally, they exhibit impressive self-healing properties. Because they are composed almost entirely of protein, their material properties must arise from specific aspects of the protein building blocks.

Current research in the group is aimed at understanding the biochemical and structural factors that provide self-healing properties to byssal threads. Results indicate that coordination cross-links between metal ions and byssal proteins in the fibrous interior of threads may function as reversibly breakable bonds that rupture prior to the covalent bonds. In doing so, these sacrificial bonds dissipate mechanical energy and spare the whole structure from catastrophic failure. Apparently, these bonds reform once the load has been removed allowing threads to heal and recover initial material properties. These chemical concepts could potentially be applied to the production of self-healing polymeric fibers.

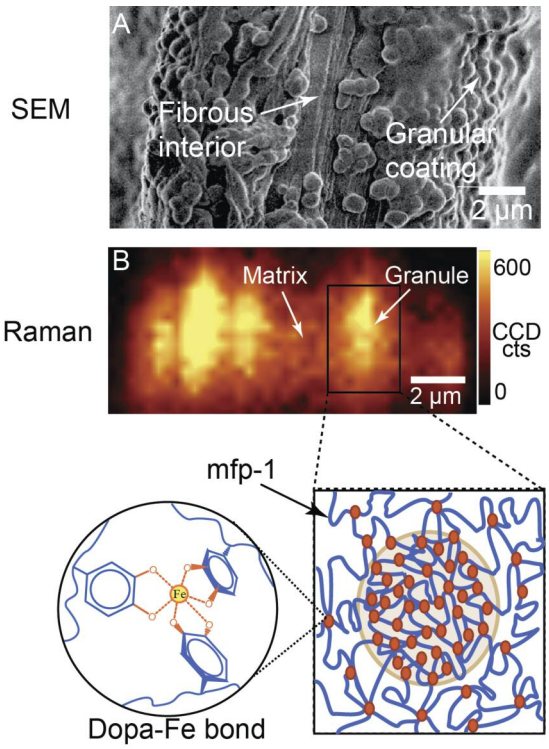

Fig. 2: (A) The outer cuticle of byssal threads is a thin and granular protective coating that uniquely combines hardness and extensibility. (B) Resonance Raman spectroscopy revealed that the cuticle consists of metallopolymeric protein (mfp-1) network with regions of high (granules) and low (matrix) dopa-Fe cross-link density. Granules thus provide cuticle hardness, whereas the matrix provides extensibility.

Prior to forming the new research group, I studied similar questions as an Alexander von Humboldt postdoctoral researcher. During this period, in collaboration with Admir Masic, I investigated the chemical composition of the outer cuticle of the byssal threads **[1]**. This thin protective coating serves to shield the fibers from abrasive damage by wave-borne debris and is interesting from a biomimetic perspective due to its remarkable combination of hardness and extensibility. *In situ* confocal Raman spectroscopic imaging revealed that the unique combination of properties arises from a non-homogenous distribution of metal coordination cross-links between iron ions and cuticle proteins (**Fig. 2**). Areas of high concentration (granules) are believed to provide the epoxy-like hardness of the material, whereas the areas of low concentration (matrix) provide the large extensibility and permit reversible crack-formation. This interaction was found to be mediated by an uncommon post-translationally modified amino acid known as 3,4-dihydroxyphenylalanine (dopa). Using Raman spectroscopy, similar dopa-Fe complexes were further identified in other regions of the thread including the attachment plaque, where they likely play a role in the adhesive properties of the byssus **[2]**.

In collaboration with researchers in the US, the biomimetically extracted chemical concepts from the byssus cuticle were adapted to synthesize metallopolymers that exhibited enhanced mechanical performance, including self-healing properties **[3]**. Specifically, dopa-Fe^{3+} crosslinks were integrated into a hydrogel network by employing a pH-dependent assembly process, mimicking the mussel thread formation process. At elevated pH, sticky gels were formed, which were able to recover initial material properties following mechanical failure. These studies provide support for the viability of such chemical level biomimetic approaches in designing and creating synthetic materials.

Additionally, in collaboration with the Plant Biomechanics group lead by Ingo Burgert, the actuation behavior of desert ice plants (Aizoaceae) was investigated in the framework of SPP-1420 program "Biomimetic Materials Research: Functionality by Hierarchical Structuring of Materials" **[4]**. The seed capsules of these plants are noteworthy because despite being non-living they undergo a complex and reversible actuated unfolding process during wetting and drying cycles (**Fig. 3A-B**). Our research found that seed capsules utilize a hierarchical arrangement of specialized swellable cells to orchestrate an Origami-like bi-directional movement in the whole structure (**Fig. 3C**). The extracted concepts from this actuated plant structure could have important implications for the growing field of "programmable matter".

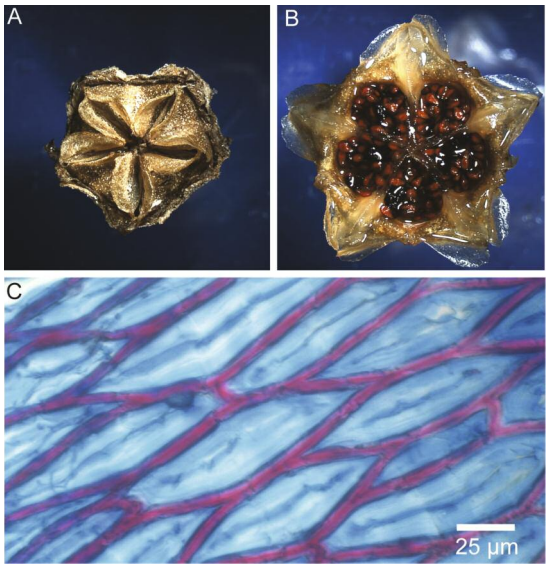

Fig. 3. (A-B) Seed capsules from the ice plant Delosperma nakurense have protective valves that unfold when hydrated. (C) Actuation of these non-living tissues is mediated by specialized cells containing a swellable cellulosic filler material.

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References:

[1] Harrington, M. J., Masic, A., Holten-Andersen, N., Waite, J. H., and Fratzl, P. (2010) Iron-clad fibers: a metal-based biological strategy for hard flexible coatings. Science 328, 216-220.

[2] Hwang, D.S., Zeng, H., Masic, A., Harrington, M.J., Israelachvili, J.N. and Waite, J.H. (2010) Protein- and metal-dependent interactions of a prominent protein in mussel adhesive plaques. Journal of Biological Chemistry 285, 25850–25858.

[3] Holten-Andersen, N., Harrington, M.J., Birkedal, H., Lee, B.P., Messersmith, P.B., Lee, K.Y.C. and Waite, J.H. (2010) Mussel-inspired metal-ligand cross-links yield polymer gels with near covalent elastic moduli and self-healing properties. Proceedings of the National Academy of Science, U.S.A. DOI: 10.1073/pnas.1015862108.

[4] Harrington, M.J., Razghandi, K., Ditsch, F., Guiducci, L., Rueggeberg, M., Dunlop, J.W.C., Fratzl, P., Neinhuis, C. and Burgert, I. Nature's actuated origami: hydro-responsive unfolding of ice plant seed capsules. submitted.

Spider Cuticle – A Study of the Structure-Function Relation

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References:
[1] Barth, F. G.: Spider Senses – Technical Perfection and Biology. Zoology **105**, 271-285, (2002).
[2] Vincent, J. F. V. & Wegst, U. G. K.: Design and Mechanical Properties of Insect Cuticle. Arthropod Struct. Dev. **33**, 187-199, (2004).
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The arthropods cuticle is a fascinating multifunctional material. It acts as a shield and a skeleton, but also comprises specialized tools and sensilla (sense organs). Its mechanical properties are thus finely tuned to stand the demands of its different functions. The main components of the cuticle are α-chitin (polyacetylglucosamine), arranged into crystalline fibrills (3nm wide, 300 nm long), and a protein matrix. The proteins adsorb on specific planes of the chitin crystals, forming higher order fibers. In spiders, these fibers form sheets which then stack in a plywood structure. The mechanical properties of the cuticle are determined by its composition, namely the chitin to protein ratio, cross-linking, and water content, and by structural factors, mainly fibers packing and orientation. Overall, these changes allow 20-fold variation in the cuticle stiffness.

Our study focuses on two extreme cuticle-based systems, each having very different mechanical demands: the vibration-sensing slit sensilla and the cheliceral-fangs used by spiders to inject prey with poison. We study the fiber arrangement at various size scales – from the molecular level to higher-order hierarchical organization – using X-ray scattering (SAXS) and diffraction (XRD) methods, electron microscopy and x-ray μCT. These findings are correlated with investigation of the mechanical properties with high spatial resolution techniques and in hydrated conditions, using scanning acoustic microscopy (SAM) and nano-indentation.

The Slit Sensilla and Lyriform Organ:
The strain sensitive slit sensilla are elongated openings in the cuticle. They are located on the legs, abdomen and chelicerae, and occur as single isolated slits or as a compound organ built of arrays of oriented slits (the lyriform organ). The slit sensilla react to minute deformations (in the order of a few Ångstoms (10-10m)) caused by internal (e.g. locomotion) or external (e.g. substrate vibration) sources, which make them one of the most sensitive sensory systems in the animal kingdom. The slit sensilla show also incredible specificity. The environmental signals are preferentially filtered such that only biologically-relevant signals are transferred to the nervous system, while other frequencies are filtered out. This allows interpretation of complex environmental signals with relatively little processing by the central nervous system. As such, the mechanosensors of spiders are a particularly interesting model for the design of materials with embedded sensory properties.

The Chelicerae
The cheliceral fangs of the wandering spider, Cupiennius salei, are curved hollow structures with a single opening of the venom canal close to the tip. The spider mostly feeds on insects, thus its fang has to puncture and cut through insect cuticle, made of similar material.The fiber orientation in the inner part of the fang is mainly parallel to its surface while in the outer part the fibers run concentrically. The outermost cuticular layer seems to contain no chitin fibers at all. In various invertebrates such as insects and worms reinforcement against wear is achieved by incorporation of transition metals e.g. Zn, Mn and Cu into the protein matrix. In others, Cu, Ca and Mg ions are deposited as various mineral forms. By means of x-ray fluorescence we have identified the presence of Zn, Mn Ca and Mg in the fangs. We also find correlation between the presence of the metal ions and increased mechanical properties of the cuticle, although the manner in which they are incorporated is still unknown. To address this question, we complement our structural study with a spectroscopic analysis, using IR and X-ray absorption spectroscopies (XANES and EXAFS).

A better understanding of the mechanisms evolved in these organisms to tailor the materials properties of the cuticle to fulfill different functions will also serve the design of sophisticated materials with desired mechanical properties and sensory functions.

The work is performed in a close collaboration of Prof. Friedrich Barth of the University of Vienna.

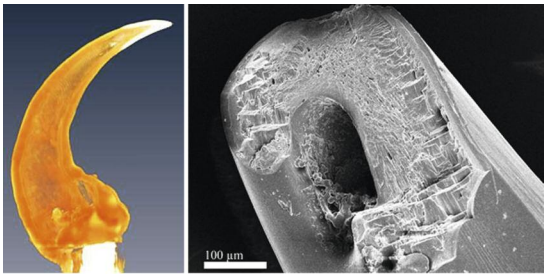

Fig. 1: The cheliceral fang of the wandering spider (a) a slice from a X-ray tomogram of the fang. The bright areas correspond to the higher density of the cuticle where the metals ions are incorporated. (b) Scanning electron micrograms of a fracture close to the tip of the chelicerae, at the opening of the venom canal.

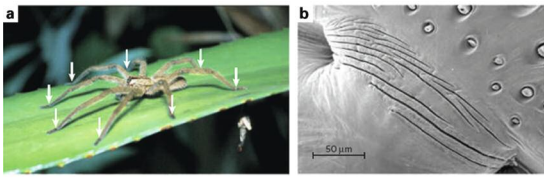

Fig. 2: Vibration-sensitive slit organ. a, Cupiennius salei, with arrows pointing to the location of the vibration sensors on the legs. B. Scanning electron micrograph of the vibration detector (dorsal view). Adapted from Ref. ***[3]***

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Calcified Cartilage in Shark Skeletons

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Calcified Cartilage in Shark Skeletons

The elasmobranch fishes (sharks, rays and relatives; **Fig. 1A**) are some of the fastest and largest animals in the ocean, and many can feed on extremely large or hard prey. This high performance is counterintuitive: the skeletons of these fishes are fashioned entirely of cartilage, which, unlike bone, is incapable of healing **[1-2]**. The morphology of this mineralized cartilage is strikingly different from that of mammals: most skeletal elements are sheathed in calcified tiles – "tesserae", each <500µm wide – covering the uncalcified cartilage underneath (**Fig. 1B,C**). This gross patterning of shark cartilage has been known for nearly two centuries, but the basic questions surrounding the material properties, micro/nanostructural organization and development of this tissue are barely addressed **[e.g. 2-4]**. A synthesis of my previous work and our current data suggests that important insight into mineralization processes can be gained by looking at chondrocytes (cartilage cells; **Fig. 1B,D**) in the mineralized and unmineralized tissues. As tessellation (tiling) is a vital feature of growth and mechanics in the skeleton **[2, 4]**, our work details the organization and functional advantages of the tissue.

The high functionality of elasmobranch cartilage is surely related to the unique arrangement of its mineralized tissue; the maintenance of the tiled pattern throughout life is therefore vital. To this end, as the skeleton grows, the number and/or size of tesserae must increase; the latter appears to be the predominant mechanism **[2]**. Tesserae grow by "engulfing" living chondrocytes from underlying uncalcified cartilage and entombing them in a highly cellular intratesseral network, interconnected by canalicular passageways analogous but considerably larger to those in bone **[5]** (**Fig. 1D,E**). Tissue samples from animals injected with a fluorescent marker for calcifying tissue (**Fig. 1F**) suggest that initial mineralization is localized around the cells themselves **[3]**. If the entombed cells are epicenters of skeletal growth and calcification (which continues throughout life); and, since deposited mineralized material cannot be removed in this tissue; the cells may be slowly "walling themselves in" by filling the space around them with permanent calcified tissue. Also, since tesserae increase in size as animals age, mineralizing material is surely distributed to and deposited at the tesseral margins. We therefore believe that the canalicular passages are critical to maintaining favorable cellular environments while aiding in distributing materials/products important to mineralization.

If these hypotheses of tissue growth are correct, these processes should be reflected in local variation in tissue properties and composition, with the degree of mineralization changing with distance from tesseral margins and from the cells themselves. Our current work supports this **[3]**: backscattered electron imaging (BEI) shows complex patterns of mineral variation in tesserae, concentric to cellular lacunae (spaces housing cells) and to tesseral edges, with tissue along the margins of tesserae and lacunae being less mineralized (**Fig. 1E**). Raman spectroscopic images of tesserae support the local variations in mineral content shown in BEI data and suggest an inverse correlation of mineral and collagen content; however these trends remain to be teased apart from effects resulting from variations in collagen fiber orientation (**Fig. 1G**). These data combined with x-ray scattering data, recently gathered at the local synchrotron facility (BESSY II), will describe the coordination and arrangement of organic and inorganic materials in tesserae. This will elucidate aspects of the framework around which the meta-pattern of skeletal "tiling" is based, perhaps providing clues to inform development of low-density, high-stiffness/high-damping engineering composites **[4]**. Our work also provides an important missing step in our understanding of skeletal tissue evolution, clarifying mineralization paradigms in the oldest surviving group of vertebrates with mineralized skeletons.

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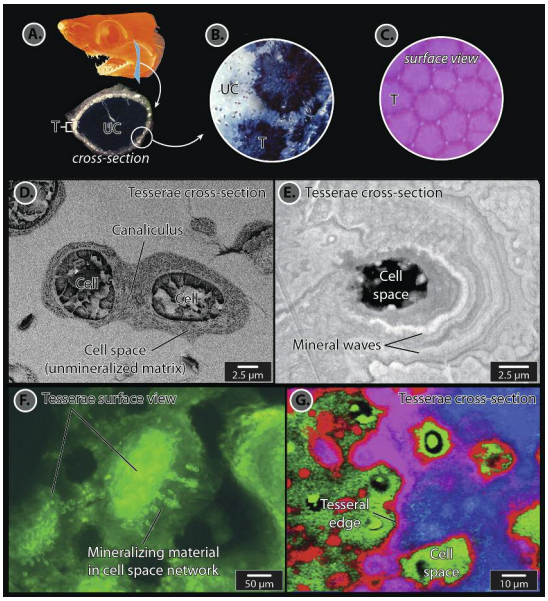

Fig. 1: The tessellated cartilage of sharks (A; lateral view of CT scan) is comprised of uncalcified cartilage, UC, overlain by a rind of calcified tiles (≤500µm wide) called tesserae, T, shown here in cross-sectional (B) and surface views (C). The cells inside tesserae (D, also visible in B) are living and connected to one another by short passageways – the waves of varying mineral density radiating out from cell spaces (E; BSE image), the movement of mineralizing tissue through the canalicular network (F; histology of oxytetracycline-injected animal), and the variation in chemical signature with distance from cells and tesseral margins (G; Raman hyperspectral image, colors represent distinct chemical spectra) provide clues cells' roles in growth mechanisms.

References:
[1] Dean, M.N. and Summers, A.P.: Cartilage in the skeleton of cartilaginous fishes. Zoology. **109**(2): 164-168 (2006).
[2] Dean, M.N., Hale, L.F., Mull, C.G., Gorb, S.N. and Summers, A.P.: Ontogeny of the tessellated skeleton: Insight from the skeletal growth of the round stingray Urobatis halleri. Journal of Anatomy. **215**(3): 227-239 (2009).
[3] Dean, M.N., Gorb, S.N. and Fratzl, P.: Ultrastructural morphology of calcified cartilage in the skeletons of sharks and rays. Gordon Research Conference – Biomineralization (2010).
[4] Liu, X., Dean, M.N., Summers, A.P. and Earthman, J.C.: Composite model of the shark's skeleton in bending: a novel architecture for biomimetic design of functional compression bias. Materials Science & Engineering C. **30**: 1077-1084 (2010).
[5] Dean, M.N., Socha, J.J., Hall, B.K. and Summers, A.P.: Canaliculi in the tessellated skeleton of cartilaginous fishes. Journal of Applied Fisheries – 'Interdisciplinary Approaches in Fish Skeletal Biology'. **26**: 263-267 (2010).

Hierarchical Structure of Biological & Biomimetic Materials

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The role of structure in biological and biomimetic materials at different length scales is crucial for their mechanical properties and biological functions.

We investigate structural arrangements of cellular and organic constituents as well as mineral phases in biological tissues to learn more about formation, function and properties of these tissues. We use high-resolution two- and three-dimensional imaging techniques from material science to characterize the evolution of the micro- and nanostructure of various tissues. In bone tissues we aim to understand dynamic processes, such as mineralization of the organic matrix as it occurs during remodeling and bone healing. By applying *in situ* micromechanical and synchrotron-based methods on biological and biomimetic materials we elucidate basic mechanisms by which the structure controls the mechanical performance.

The Organization of the Osteocyte Network Mirrors the Extracellular Matrix Orientation in Bone

Bone is constantly undergoing remodeling as a result of the interplay between bone resorption by osteoclasts and bone formation by osteoblasts. During bone formation some of the osteoblasts are embedded in the mineralizing collagen matrix and differentiate to osteocytes, forming a network throughout the whole bone tissue.

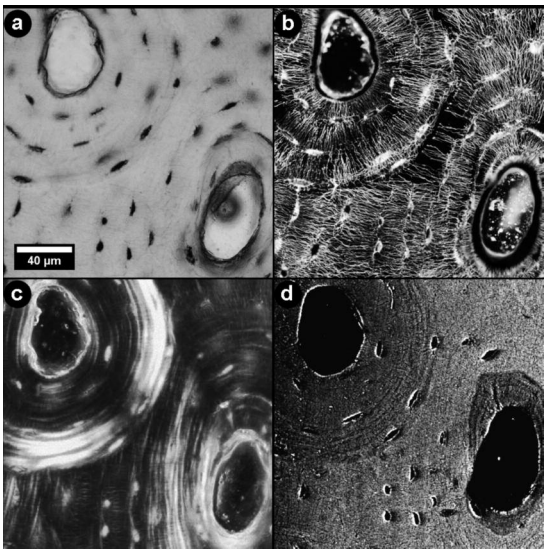

Fig. 1: Equine metacarpal bone: osteons, embedded in older bone matrix; (a) area visualized by light microscopy, (b) confocal microscopy, (c) polarized microscopy and (d) backscattered electron microscopy.

We investigated the extent to which the organization of the osteocyte network correlates with the collagen matrix arrangement in different bone tissues **[1]**. Several tissue types -with different degrees of organization- from equine, ovine and murine bone have been examined using three-dimensional imaging of osteocyte networks by confocal laser scanning microscopy. Furthermore, the tissues have been characterized by polarized light microscopy and back-scattered electron imaging (**Fig. 1**).

The spatial arrangement of unorganized and organized bone is shown schematically in **Fig. 2**. We conclude that osteoblasts synthesize and utilize scaffold-like tissue as a guide for the deposition of highly ordered and mechanically competent bone tissue by a collective action of many cells. The collective cell action is facilitated by a substrate layer whose surface directs this process. Without this substrate osteoblasts build microlamellar bone which is then used as a substrate layer.

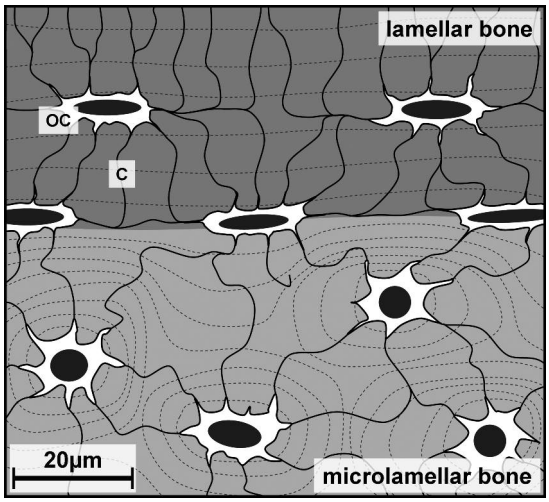

Fig. 2: Scheme of the osteocytic network and corresponding matrix orientation (red dashed lines). Top: lamellar bone layer with highly aligned osteocytic network; alignment of lacunae (OC) is parallel; canaliculi (C) run mainly perpendicular to the lamellae. Bottom: microlamellar bone with lower degree of organization; canaliculi run mainly radially from osteocytes.

Furthermore, we have qualitatively shown that primary bone in the callus of sheep bone has a low degree of order in cell organization and in collagen architecture **[2]**. Similar to formation of new bone, in fracture healing secondary lamellar bone is deposited on top of this scaffold-like primary structure.

In the context of bone tissue engineering, this encourages the idea that scaffolds mimicking the primary bone architecture may be developed to accelerate bone regeneration, for example in critical defect healing.

Microstructural Properties and Growth of Antler Bone

Bone appears in many forms, fulfilling numerous mechanical functions. One example of a particular tough bone material is deer antler. This is an exceptional biomineralized organ which is completely regenerated every year and is used as a fighting weapon by rival male deer.

We used time-resolved synchrotron small angle X-ray diffraction together with tensile testing of antler bone to elucidate the structural origin of the antler's high toughness **[3]**. It has been shown for other bone types **[4]** as well as synthetic materials, such as shape-memory polymers **[5]**, that *in situ* tensile testing in combination with X-ray diffraction is an appropriate tool to investigate deformation mechanisms simultaneously at different levels of hierarchy. The deformation at the nanoscale (fibril strain) could be determined from changes in the diffraction pattern during macroscopic tensile tests (tissue strain). The results show that in deer antler on average fibrils are strained only half as much as the whole tissue and the fibril strain increases linearly with tissue strain, both during elastic and inelastic deformation.

Fig. 3 shows the change in average strain (black circles) of the fibrils with increasing macroscopic tissue strain. The distance between upper and lower curves (white circles) is an indication of the width of the fibril strain distribution. During elastic deformation all curves rise linearly (same slope) with tissue strain implying all fibrils are stretched to the same extent. Most remarkably, beyond a certain tissue strain different fibrils start to show increasingly different degrees of elongation at the same macroscopic strain. This strain-inhomogeneity on the fibril level increases with tissue strain beyond the yield as indicated by the shaded region in **Fig. 3**. The average fibril strain rises linearly until sample failure. This inhomogeneous fibrillar strain pattern at the nanoscale may explain the extreme toughness of antler compared to normal bone.

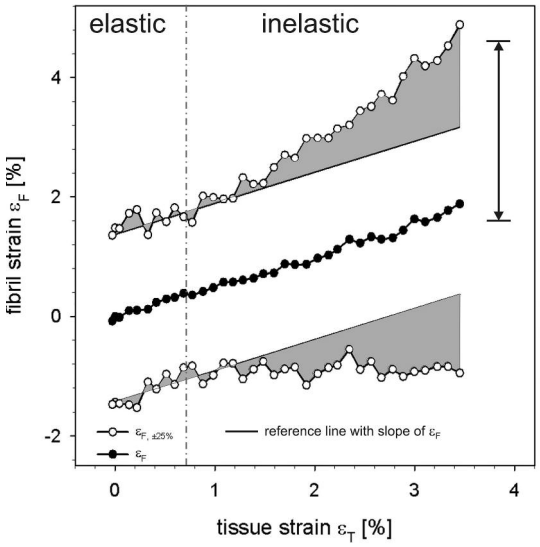

Figure 3: Average fibril strain ε_F (black circles) in antler bone plotted against tissue strain ε_T as determined from in situ x-ray tensile tests. Upper and lower limits of the fibril strain distribution $\varepsilon_{F,\pm25\%}$ within the X-ray scattering volume are plotted as white circles. The average fibril strain ε_F increases linearly in both the elastic and inelastic range. The bidirectional arrow on the right displays the mean fibril strain range at 100% mean macroscopic fracture strain.

Further studies on antler, looking at the extremely fast growth from a material-design-perspective, show that antler bone growth takes place via at least two scaffold structures, the mineralized cartilage and the bone framework respectively. These scaffolds are characterized by a highly anisotropic tubular architecture. Maturation of antler cortical tissue occurs by directed bone ingrowth into the bone framework: a highly mineralized, lamellar bone matrix with varying fiber orientations is filled by less mineralized, longitudinally oriented bone rods (primary osteons).

This material design, containing interfaces between structural features with different properties, may also lead to an increase in toughness and prevent crack initiation and propagation.

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References:

[1] Kerschnitzki, M., Wagermaier, W., Roschger, P., Seto, J., Shahar, R., Duda, G.N., Mundlos, S., Fratzl, P.: The organization of the osteocyte network mirrors the extracellular matrix orientation in bone. J. Struct. Biol., **173**, 303-311 (2011).

[2] Kerschnitzki, M., Wagermaier, W., Liu, Y., Roschger, P., Duda, G.N., Fratzl, P.: Primary bone as an endogenous scaffold for the deposition of secondary lamellar tissue in rapidly growing ovine bone. Cells Tissues Organs, DOI: 10.1159/000324467, (2011).

[3] Krauss, S., Fratzl, P., Seto, J., Currey, J. D., Estevez, J. A., Funari, S.S., Gupta, H. S.: Inhomogeneous fibril stretching in antler starts after macroscopic yielding: indication for a nanoscale toughening mechanism. Bone, **44**(6), 1105-1110, (2009).

[4] Gupta, H.S., Seto, J., Wagermaier, W., Zaslansky, P., Boesecke, P., Fratzl, P.: Cooperative deformation of mineral and collagen in bone at the nanoscale. Proc. Natl. Acad. Sci. U S A, **103**(47):17741-6, (2006).

[5] Wagermaier, W., Zander, T., Hofmann, D., Kratz, K., Kumar, U.N., Lendlein, A.: In Situ X-Ray Scattering Studies of Poly(ε-caprolactone) Networks with Grafted Poly(ethylene glycol) Chains to Investigate Structural Changes during Dual- and Triple-Shape Effect. Macromol Rapid Comm, **31**(17), 1546-1553, (2010).

Plant Biomechanics and Biomimetics

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The Plant Biomechanics and Biomimetics research group is interested in the structure-function-relationships of plants mainly at the micro-and nanoscale. We intend to understand the underlying principles of how plants accomplish their excellent mechanical performance and how tissues are pre-stressed during growth. The gained knowledge allows for a deeper insight into plant strategies to mechanically adapt to environmental conditions as well as the mechanisms of plant movements, for instance for effective seed dispersal. The examined biological systems are valuable sources for extracting biomimetic principles as the specific functionality is achieved by a clever structuring which turns even dead plant tissues into active and responsive devices.

The group research profile comprises three main activities; the analyses of structure and properties of plant cell walls, the generation of stresses and plant movements as well as projects towards a biomimetic transfer of the unravelled principles. The methods utilized are tensile tests to investigate tissue and fibre mechanical properties in combination with (nano)structural examination techniques such as X-ray scattering, Raman spectroscopy, Environmental Scanning Electron microscopy and cryo-SEM.

Cell Wall Structure and Properties

The plant cell wall represents a fibre-reinforced polymer assembly of stiff cellulose fibrils of a few nanometers in diameter, embedded in soft matrix macromolecules (hemicelluloses, pectins, lignin, structural proteins). The mechanical design of this nano-composite is analyzed, in order to gain better insights into optimization strategies of living plants as well as into the material design of cell walls **[1]**. These investigations are conducted on thin and flexible primary cell walls which allow the living cell to grow and on thick and rigid lignified secondary cell walls which mechanically stabilize the plant body. Both cell wall systems are investigated in both the natural condition as well as in a genetically modified state. This is done in close collaboration with colleagues from the fields of plant physiology, biochemistry and molecular biology. Collaboration exists on campus with the MPI for Molecular Plant Physiology (MPI-MP), Potsdam as well as with partners from the recently expired EU-Project CASPIC.

In terms of primary cell walls mechanical analysis was performed on Arabidopsis hypocotyls of various transgene lines and chemical pre-treatments, each affecting one of the crucial cell wall components. Plants with modification in xyloglucan structure were investigated together with the Markus Pauly Lab (Berkeley). In collaboration with Herman Höfte (INRA-Versailles) we studied modified Arabidopsis plants with alterations in the protein structure of the cellulose synthase complexes and the pectin composition **[2]**. The mechanical performance of Arabidopsis plants with alterations in the cytoskeleton and/or in the cellulose synthase complexes was investigated together with Staffan Persson from the MPI-MP.

In terms of secondary cell walls most research activities were based on transgene aspen plants which were examined in collaboration with the lab of Björn Sundberg, Plant Science Center, Umea, Sweden. Here we investigated how the genetic modification affected cell wall nanostructure and mechanical performance. Besides micro-mechanical tests, the cellulose fibril orientation and cellulose fibril/ matrix interactions were investigated by X-ray scattering and Raman microscopy. Studies on a mutant with a lignin content reduced by ~30% showed only minor differences in tensile properties compared to the wildtype **[3]**.

In de- and rehydration experiments, combined with simultaneous X-ray diffraction measurements and tensile straining, we investigated together with Peter Fratzl how moisture changes below the fibre saturation point affect the crystal lattice of the cellulose fibrils in the wood cell wall (**Fig. 1**).

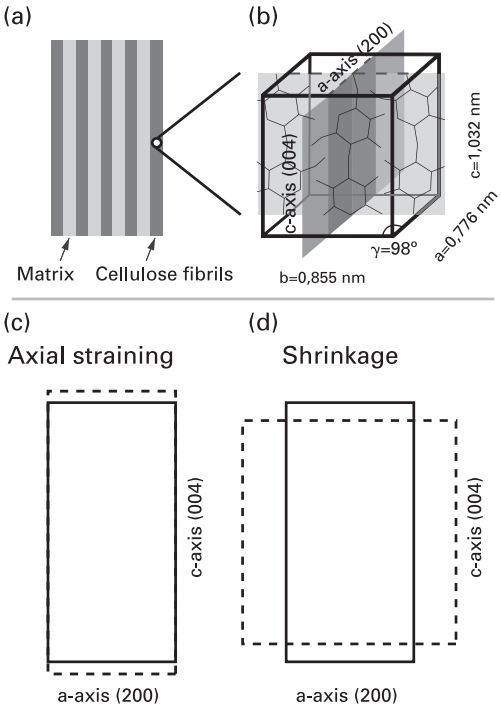

*Fig. 1: Schematic of (a) the alternating cellulose fibril – matrix structure in the cell wall and (b) of the axis of the crystal structure of cellulose. Deformation of the crystal lattice of cellulose due to (c) axial tensile loading and (d) drying **[4]**.*

Axial stresses resulted in a longitudinal expansion without significant changes in the transverse direction whereas during drying a longitudinal contraction and transverse expansion of the crystalline cellulose was measured. In view of the high stiffness of crystalline cellulose, the magnitude of the deformation can not be explained by stresses generated by the shrinking matrix molecules. It is more likely that water adsorption and desorption at the cellulose surface accounts for the moisture dependent changes in the dimensions of the cellulose crystal lattice **[4]**.

Further studies on structure-function-relationships in plants were aiming at understanding the basic principles of the organization of stiffness gradients between tissues. Here we studied the gradient transitions in structure and mechanical properties of individual wood cells across a growth ring of spruce **[5]** and the design of structural and mechanical interfaces between tissues in the giant reed (*Arundo donax*) **[6]**.

Stress Generation and Plant Movement

Research on the mechanisms of stress generation and plant movement is conducted in close cooperation with Peter Fratzl **[7]**.

After having studied stress generation in tension wood of hardwoods, we examined in a recent study the root contraction in perennial plants. Also for the roots of red clover we found tension wood fibres with the characteristic gelatinous layer (G-layer) filling the lumen of the cells. Raman microscopy studies and measurements of tissue deformation after enzymatic removal of the G-layer suggested that the mechanism of root contraction is similar to stress generation in tension wood of poplar **[8]** (**Fig. 2**).

However, tension wood of trees is optimized for generating high stresses and small deformation whereas contractile roots need to be largely deformed with little stresses involved. Investigations on how the system is structurally fine-tuned to be able to fulfill both opposed functions are ongoing.

In the framework of the SPP 1420 we run a project on the unfolding mechanism of ice plant seed capsules in collaboration with Christoph Neinhuis (TU-Dresden). These capsules show a complex opening mechanism upon wetting for seed dispersal. Interestingly also in this plant a gelatinous layer was found. Here, the highly swellable layer forces the cells to deform almost exclusively in one of the transverse directions which enables such large deformations of the valves upon wetting (see also report by Matt Harrington).

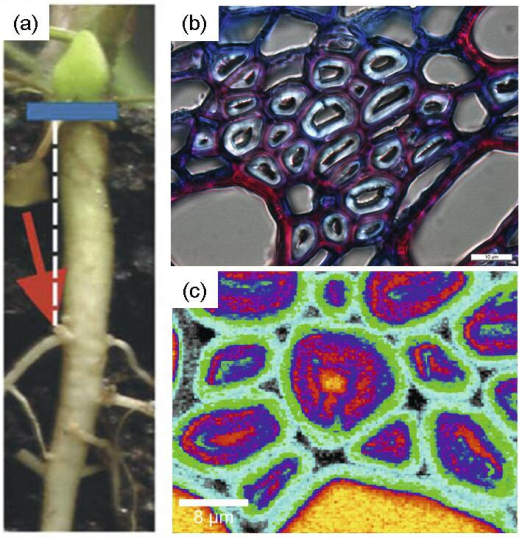

Fig. 2: (a) Contractile root of red clover at an age of 13 weeks (b) cross-section with tension fibres containing the blue-stained G-layer in the lumen, (c) Raman microscopy image of water content showing that the G-layer in the lumen (red colour) contains more water than the surrounding cell wall layers (green colour) ***[8]****.*

Bio-Inspired Materials

In two projects in collaboration with partners from the Departments of Colloid Chemistry and Interfaces as well as the universities of Freiburg and Bayreuth and the ITV-Denkendorf we are aiming at transferring the design principles of plant cell walls to synthetic systems. In the first project we have produced anisotropic Agarose hydrogels by embedding cellulose nanowhiskers and orienting them through external tensile straining. In the second project we intend to develop innovative glass fibre composites in the framework of a BMBF project. Here the group of Helmut Schlaad has developed a synthesis strategy which allows binding polymer chains of various lengths to the glass fibre surface. In single fibre pull out tests, conducted in our lab, we investigate whether the toughness of the fibre-reinforced composite can be increased by this approach. Further a review article about bio-inspired materials research has been published **[9]**.

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References:

[1] Salmén, L., Burgert, I.: Cell wall features with regard to mechanical performance. Holzforschung **63**, 121-129 (2009).

[2] Abasolo, W., Eder, M., Yamauchi, K., Obel, N., Reinecke, A., Neumetzler, L., Dunlop, J.W.C., Mouille, G., Pauly, M., Höfte, H., Burgert, I.: Pectin may hinder the unfolding of xyloglucan chains during cell elongation – implications of the mechanical performance of Arabidopsis hypocotyls with pectin alterations. Mol Plant **2**, 990-999 (2009).

[3] Bjurhager, I., Olsson, A.-M., Zhang, B., Gerber, L., Kumar, M., Berglund, L., Burgert, I., Sundberg, B., Salmén, L.: Ultra-structure and mechanical properties of Populus wood with reduced lignin content caused by transgenic down-regulation of cinnamate 4-hydroxylase. Biomacromolecules **11**, 2359-2365 (2010).

[4] Zabler, S.A., Paris, O., Burgert, I., Fratzl, P.: Moisture changes in the plant cell wall force cellulose crystallites to deform. J Structural Biol. **171**, 133-141 (2010).

[5] Eder, M., Jungnikl, K., Burgert, I.: A close-up view of wood structure and properties across one growth ring of Norway spruce (Picea abies [L.] Karst.). Trees **23**, 79-84 (2009).

[6] Rüggeberg, M., Burgert, I., Speck,T.: Structural and mechanical design of tissue interfaces in the Giant Reed (Arundo donax). J. R. Soc. Interface **7**, 499-506 (2010).

[7] Burgert, I., Fratzl, P.: Actuation systems in plants as prototypes for bioinspired devices. Phil. Trans.R. Soc.A **367**, 1541-1557 (2009).

[8] Schreiber, N., Gierlinger, N., Pütz, N., Fratzl, P., Neinhuis, C., Burgert, I.: G-fibres in storage roots of Trifolium pratense (Fabaceae): Tensile stress generators for contraction. The Plant Journal **61**, 854–861 (2010).

[9] Paris, O., Burgert, I., Fratzl, P.: Biomimetics and biotemplating of natural materials. MRS Bulletin, Special Issue, Materials from Renewable Resources **35**, 219-225 (2010).

Biomimetic Actuation and Tissue Growth

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Compared to an Engineer, who can access the entire periodic table, Nature uses only a limited palette of elements to make structural materials. Despite this, a wide range of material properties is achieved by combining these elements into a composite material **[1, 2]**. Many of these natural materials are fibrous composites, in which the material properties are controlled precisely by the architectural arrangement of the fibres. On one hand it is important to understand the role of architecture on the resultant material properties and on the other hand how a particular tissue architecture is achieved during growth. These two themes are investigated in this group through three different topics: 1) by understanding the link between hygroscopic actuation in plant organs and the underlying tissue architecture (with I. Burgert, Plant Biomechanics Group, R. Elbaum and Y. Abraham, Hebrew Uni. Jerusalem, Y. Bréchet, INP-Grenoble and T Antretter, Uni. Leoben), 2) through the analysis of tissue orientation in the early stages of bone fracture healing (with A. Masic, Biomaterials Department and G. Duda at the Charité-Berlin); and 3) by studying how substrate geometry controls tissue growth (with, M. Rumpler, Boltzmann Institute, Vienna, F. D. Fischer, and E. Gamsjäger Uni. Leoben). The three themes share a common thread in that they deal with understanding the mechanics of shape and volume changes in fibrous tissues.

Controlling Actuation Through Architecture

Many plants have organs that can move in complex ways, controlled by a clever organisation of their underlying tissues. Examples include the awns of wheat or the stalksbill which are used to help propel seeds along and into the ground, and the opening of the scales on pine-cones or the valves on the ice-plant to allow for seed release (see I. Burgert and M. Harrington – Plant Biomechanics). In all of these systems actuation occurs in dead tissue which swells/contracts in controlled ways due to changes in external humidity. As these systems require no active energy transport by the plant, it is therefore the architecture of the tissue which gives rise to the complex movements. Our goal therefore is to understand the link between the architecture arrangement of swellable tissues and the resultant motion. A simple example of the effect of architecture on movement is given in **Fig. 1**. Here simple composite beams with a uniform cross-section have been simulated. The beam consists of two materials, active and swellable (grey) and passive and non-swellable (white), distributed uniformly along the beam, however with different symmetric arrangements within the cross sections (top row). Results of finite element simulations (bottom row) demonstrate that the symmetry of motion is controlled by the symmetry of the tissue architecture. Even if the materials are arranged only in two dimensions a large variety of three dimensional responses can still be achieved.

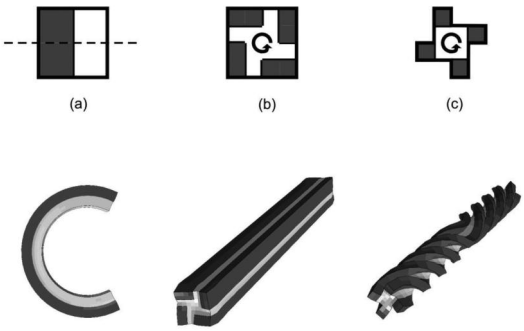

*Fig. 1: Three examples of how different arrangements of active (grey) and passive (white) materials over a cross section of a beam can influence the resultant actuation behaviour, leading to a) bending, to b) no macroscopic response, to c) twisting **[3]**.*

Collagen Organisation in Fracture Healing

The healing of fractured bone is a complex process in which an organised architecture of mineralised collagen fibrils is formed within the fracture gap. Recent work (Y. Liu Dept. Biomaterials) suggests that the formation of mineralised tissue during bone healing occurs in two "waves", an initial wave in which a scaffold of unorganised bone is quickly formed upon which a second wave of more organised lamellar bone is then produced. One issue that is still not understood is how the first scaffold of woven bone is produced and organised during the initial phases of healing when the tissue is not yet mineralised. We together with A. Masic (Biomaterials) and G. Duda (Charité) are currently investigating this in fracture calluses of rats, using the powerful technique of polarised Raman microspectroscopic imaging **[4]**. This technique allows us to produce maps of the spatial distribution and orientation of particular chemical groups such as collagen and mineral (**Fig. 2**). The initial data suggests that even in the early stages of healing the tissue becomes highly oriented, leading to an organised structure which is then the scaffold for the "second wave".

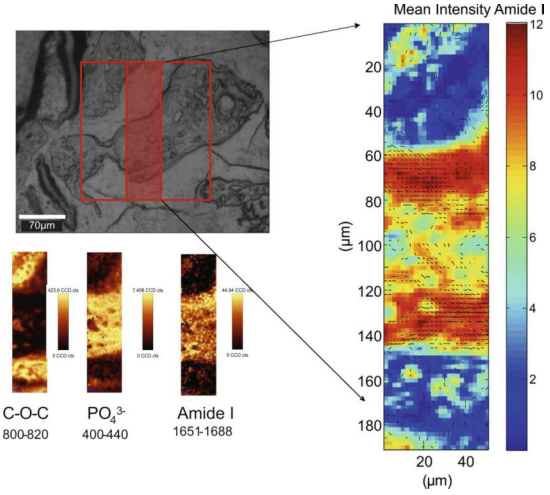

Fig. 2: Polarised raman imaging of mineralised cartilage in the rat fracture callus. The top left image is an optical micrograph of the region studied. The bottom images show chemical maps (unpolarised) of embedding material, mineral and collagen respectively. The black lines in the image on the right show the collagen orientation in the cartilage.

Geometric Control of Tissue Growth

An important focus of the group is towards gaining a more fundamental understanding of how growing tissues organise themselves inside a scaffold. By using rapid-prototyping to produce scaffolds of controlled shapes and sizes, we have shown that the tissue growth rate inside a pore correlates with the local curvature of the substrate **[5]**. This curvature controlled growth is similar to phase transformations in physics where surface tension plays an important role. In addition, it was also demonstrated that this rate of growth becomes independent of the material properties of the underlying substrate after the first cell layers were formed **[6]**, highlighting the importance of geometry on growth. In order to understand this more deeply we combine cell culture experiments with numerical simulations at different levels of complexity.

A hint towards understanding the mechanisms underlying geometric controlled tissue growth is found in the organisation of the actin cytoskeleton in tissue grown inside circular and square pores (**Fig. 3**). The actin stress filaments are aligned with the tissue border and are more concentrated close to the tissue surface, highlighting the role mechanical stresses play on growth. With this in mind we have developed a continuum model for tissue growth in confined geometries (with F. D. Fischer and E. Gamsjäger, Univ. Leoben) **[7]**. In this model a growth law consistent with the second law of thermodynamics was derived using Onsager's principle of maximum dissipation and applied to simple pore geometries. The model reproduces the observed dependence of the growth kinetics on the sign of curvature only when an explicit surface stress is introduced.

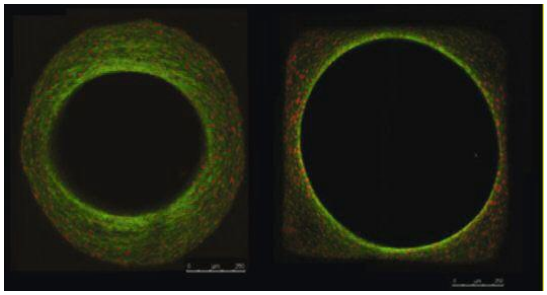

Figure 3: Confocal microscopy images of the actin cytoskeleton (green) and nuclei (red) after tissue growth by mouse pre-osteoblast cells (MC3T3-E1). Note the organisation of the actin filaments and the preferential tissue growth in the pore corners.

The fundamental understanding of the mechanisms of tissue growth has important implications in bone remodelling **[8]**, bone healing as well as in scaffold design for tissue engineering. This is highlighted in **Fig. 4** which shows the measured growth kinetics in square and cross shaped pores. By simply changing the pore geometry it was possible to accelerate tissue growth by a factor two without the addition of any other growth factors. The concept that local curvature determines growth was implemented into a numerical simulation based on actual images of the scaffolds to predict the time course of the projected tissue area. Although the growth behaviour is described well for early growth stages, by this curvature driven growth model (**Fig. 4**), further work is required to characterise and model the response of individual cells to their mechanical/geometrical environment.

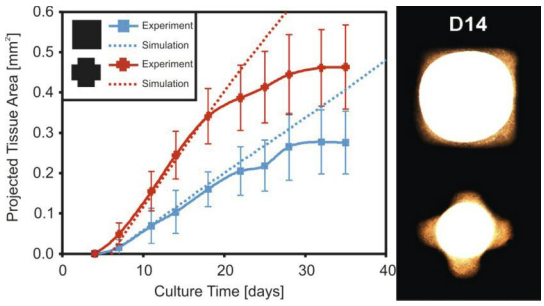

Figure 4: Tissue growth kinetics (left) measured in different shaped pores (right) compared to a simple curvature driven growth model (dotted lines).

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References:

[1] Dunlop, J. W. C. and P. Fratzl: Biological Composites. Ann. Rev. Mat. Res., **40**, 1-24 (2010).

[2] Dunlop, J. W. C., Y. J. M. Bréchet: Architectured Structural Materials: A Parallel Between Nature and Engineering. MRS Proc., v1188, 1188-LL09-04 (2009).

[3] Turcaud, S., L. Guiducci, P. Fratzl, Y. J. M. Bréchet, J. W. C. Dunlop: An excursion into the design space of biomimetic architectured biphasic actuators. submitted to Int. J Mater Res. (2010).

[4] Gamsjäger, S., A. Masic, P. Roschger, M. Kazanci, J. W. C. Dunlop, K. Klaushofer, E. P. Paschalis, P. Fratzl: Evolution of cortical bone orientation and composition as a function of bone maturation in a mouse animal model obtained by Raman spectroscopy. Bone, **47**, 392-399 (2010).

[5] Rumpler, M., A. Woesz, J.W. C. Dunlop, J. van Dongen, P. Fratzl: J. Roy. Soc. Int., **5**, 1173-1180, (2008).

[6] Kommareddy, K. P., C. Lange, M. Rumpler, J. W. C. Dunlop, I. Manjubala, J. Cui, K. Kratz, A. Lendlein, and P. Fratzl: Two stages in three-dimensional in vitro growth of tissue generated by osteoblast-like cells. Biointerphases, **5**, 45-52, (2010).

[7] Dunlop, J. W. C., F. D. Fischer, E. Gamsjäger, P. Fratzl: A theoretical model for tissue growth in confined geometries. J. Mech. Phys. Solids, **58**, 1073-1087, (2010).

[8] Dunlop, J. W. C., M. A. Hartmann, Y. J. M. Bréchet, P. Fratzl, R. Weinkamer: Bone remodeling is controlled by an activation barrier, not by a lazy zone. Calcif. Tiss. Int., **85**, 45-54, (2009).

Magnetite and Hierarchical Systems

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Nature not only provides inspiration for designing new materials but also teaches us how to use interparticle and external forces to structure and assemble these building blocks into functional entities. Magnetotactic bacteria (**Fig. 1**) and their chain of magnetosomes represent a striking example of such an accomplishment where a simple living organism precisely tunes the properties of inorganics that in turn guide the cell movement thereby providing an energetic advantage vs. the non-magnetotactic counterparts **[1]**.
In my group, we have developed a bio-inspired research based on magnetotactic bacteria. This research combines the recent developments of nanoscale engineering in the chemical science and the latest advances in molecular biology. Thereby we have created a novel methodology enabling first, the understanding of the control of biological determinants over single inorganic building blocks at the nanoscale and over highly-organized hierarchical structures, and second, the use of these biomacromolecules to construct new functional materials.

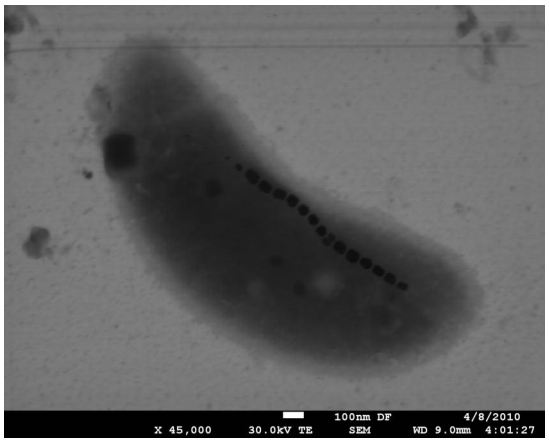

Fig. 1: a typical STEM image from a magnetotactic bacterium (strain AMB-1). The magnetosomes are the electron-dense particles that are aligned and form chain in the cells.

Biological Materials

Magnetosomes: Hierarchy at the Structural Level

The biomineralization of magnetite inside the magnetosome organelle together with the chain formation in magnetotactic bacteria are two processes that are highly controlled at the cellular level in order to form cellular magnetic dipoles. The smallest building block of this assembly is the magnetosome crystal. However, only controversial results about its microstructure were obtained so far, and the influence of the ultrastructure in the formation of the magnetic dipole i.e. on the function of the assembly is to be specified. We have thus investigated the structure of the magnetosomes using high-resolution synchrotron X-ray diffraction at the microspot beamline of the BESSY II synchrotron of the Helmholtz Zentrum Berlin **[2]**. Significant differences in lattice parameter were identified between intracellular magnetosomes from cultured magnetotactic bacteria and isolated ones (**Fig. 2**). Through comparison with abiotic control materials of similar size, we showed that this difference could be associated to different oxidation states and that the biogenic nanomagnetite was stoichiometric, i.e. structurally pure, whereas isolated magnetosomes were slightly oxidized. The hierarchical structuring of the magnetosome chain thus starts with the formation of structurally pure magnetite nanoparticles that in turn might influence the magnetic property of the magnetosome chains.

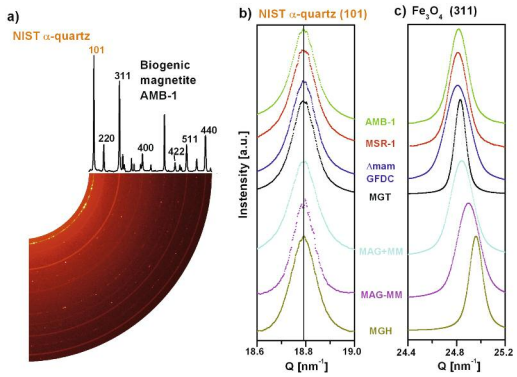

*Fig. 2: **a)** Exemplary 90° sector of AMB-1 diffraction pattern to visualize the azimuthal integration. Analyzed magnetite peaks and calibration peak (NIST α-quartz) indexed **b)** α-quartz (101) calibration peaks of different biogenic and abiotic magnetite/ maghemite samples. All peaks calibrated to Q = 18.7910nm^{-1} **c)** most intensive (311) reflex of all analyzed samples. Remarkable peak shift bet-ween biogenic magnetite in cell solution samples (AMB-1, MSR-1 and ΔmamGFDC) compared to isolated magnetosomal magnetite with and without membrane (MAG+MM and MAG-MM) and inorganic magnetite (MGT) or even more pronounced with maghemite (MGH).*

Magnetosomes Chains: Hierarchy at the Chain Level

Magnetotactic bacteria benefit from their ability to form cellular magnetic dipoles by assembling stable single-domain ferromagnetic particles in chains as a means to navigate along Earth's magnetic field lines on their way to favourable habitats. After studying the smallest building-blocks, i.e. the magnetosomes and their ultrastructure, we studied their assembly with FORC diagrams and ferromagnetic resonance

spectroscopy in order to again find how the chain can function as an entity **[3, 4]**. Magnetospirillum gryphiswaldense was cultured in a time-resolved experimental setting. Our data showed first that magnetic particle growth was not synchronized. Moreover, we could also show that the increase in particle numbers was insufficient to build up cellular magnetic dipoles. Finally dipoles of assembled magnetosome blocks occurred when the first magnetite particles reached a stable single-domain state. These stable single-domain particles could act as magnetic docks to stabilize the remaining and/or newly nucleated superparamagnetic particles in their adjacencies (**Fig. 3**). We thus could postulate that docking was a key mechanism for the building of the functional cellular magnetic dipole, which in turn controls the cells orientation.

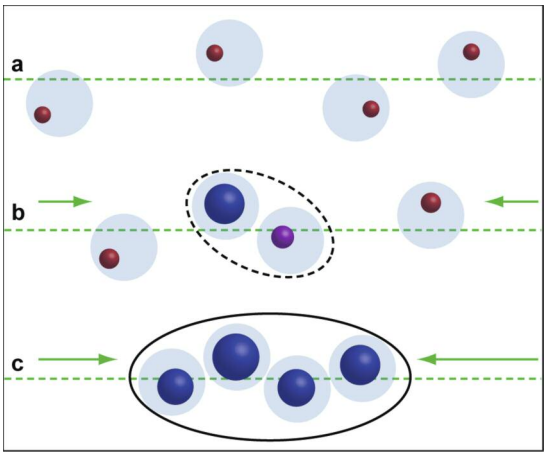

Fig. 3: Schematic sequence of cellular magnetic dipole formation. Superparamagnetic (SPM) magnetite particles (red) are nucleated in widely-spaced organelles (light blue), the green arrows indicate the biologically-driven movements of the magnetosomes along the cytoskeletal filament (dashed line) (a), stable single domain magnetite (blue dot) and its magnetic interaction (dashed-lined ellipse) act as "magnetic dock" to stabilize SPM particle (purple dot) (b). Spacing and size of magnetite are optimized, and the closed-neighbored magnetite particles separated only by magnetosome membrane generate a robust cellular magnetic dipole (solid-lined ellipse) (c). (from Faivre et al. (2010))

Biomimetic Materials

Synthetic Magnetite Nanoparticles: Studying the Initial Stages of Mineral Formation

Multiple synthetic routes for the production of magnetite nanoparticles have been reported in the literature. Indeed, the ferrimagnetic properties of such particles are increasingly exploited in bio- and nanotechnological applications. However, the formation mechanism has remained unclear. Crystallization pathways involving precipitation from soluble iron species or solid state transformations have been proposed. We have developed a set-up for the controlled formation of magnetite *in vitro* (**Fig. 4**) **[5]**. We are currently using high-resolution cryo-transmission electron microscopy to unravel the mechanism of such a formation. Our initial results indicate that magnetite forms from gradually transforming iron oxyhydroxide precursor clusters. These clusters build up colloidal crystalline assemblies, which fuse and become single or polycrystalline magnetite nanoparticles. These results are an essential step forward in the understanding of crystal formation in synthetic, geological and biomineralizing systems and will enable us to proceed to the next step of magnetite formation in the presence of biological determinant, i.e. additives found in magnetotactic bacteria.

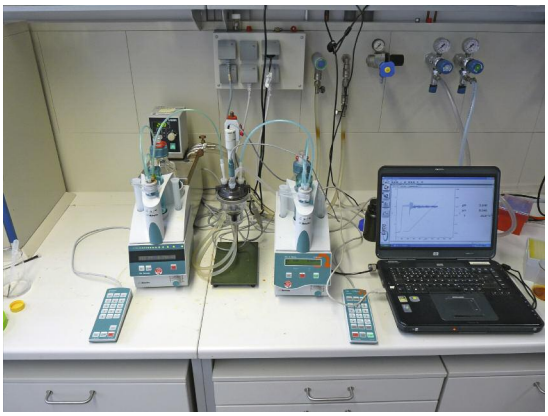

Fig. 4: set-up for the biomimetic syntheses.

Biomimetic Chains: Hierarchy in a Synthetic System

Hierarchical structuring of single particles can lead to the formation of multifunctional materials **[6]**. We are thus are interested in the biomimetic arrangement of the magnetic particles we form *in vitro* in a project funded within the 1420 Priority Programme of the DFG. MamK is a filamentous Actin-like magnetosomal protein sharing significant homology with bacterial cytoskeletal proteins such as MreB and ParM. Understanding the functionality of MamK is predicted to be critically important to the integrity of the crystal chains during *in vitro* biomimetic assembly. Cloning, over-expression and isolation of MamK are currently underway to aid physical patterning of the biomimetic nanoparticles.

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References:

[1] Faivre D. and Schüler D., Magnetotactic Bacteria and Magnetosomes. Chem. Rev. **108**, 4875-4898 (2008).
[2] Fischer A., Schmitz M., Aichmayer B., Fratzl P., and Faivre D., Structural Purity of Magnetite Nanparticles in Magnetotactic Bacteria. J. R. Soc. Interface. In press.
[3] Faivre D., Fischer A., Garcia-Rubio I., Mastrogiacomo G., and Gehring A. U., The Development of Cellular Magnetic Dipoles. Biophys. J. **99**, 1268-1273 (2010).
[4] Carvallo C., Hickey S., Faivre D., and Menguy N., Formation of Magnetite in Magnetospirillum gryphiswaldense studied with FORC diagrams. Earth Planets Space **61**, 143-145 (2009).
[5] Baumgartner J. and Faivre D., Process for Preparing Magnetite or Maghemite Nanoparticles with Controlled Size Using Mild Conditions. European Patent (2010).
[6] Faivre D., Multifunctional Materials: Dry but Flexible Magnetic Materials. Nat. Nano. **5**, 562-563 (2010).

Bio-Inspired Hybrid Materials and Synchrotron Research

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Biominerals are our source of inspiration for the design of new hybrid materials. Showing a great diversity in their structure and composition, biominerals can fulfill many different functions, ranging from load bearing and wear resistance to magnetic and optical sensing. Important concepts found in biominerals used for mechanical purposes are a large amount of organic-inorganic interfaces and structural hierarchy **[1]**. The formation of biominerals is to a large degree controlled by organic molecules. In this context, we studied the self-assembly of amelogenin proteins and their role in the biomineralization of tooth enamel. We found evidence for an oblate shape of amelogenin nanoparticles in suspension. The anisometric shape helps to explain the formation of previously observed anisotropic higher order structures which are believed to play a critical role in regulating the parallel arrangement of the hydroxyapatite crystals in enamel **[2]**. In addition to the formation of biominerals, we investigate their structure, stability and mechanical properties. Examples from crayfish and a mollusk shell are given in the following paragraphs. Our research is mainly focused on the lower hierarchical levels, i.e. the nanostructure and lattice structure. The most important questions that we address are which mechanisms contribute to stabilize amorphous biominerals and how organic molecules can get incorporated into mineral crystals. Comparative studies on bio-inspired model systems allow for deriving general concepts which can in the future help to develop new materials for technical applications. Our main experimental methods are synchrotron small- and wide- angle X-ray scattering (SAXS and WAXS), in particular at the MPI's experimental stage (μ-Spot beamline) of the BESSY II storage ring (Helmholtz-Zentrum Berlin).

Calcium Carbonate and Phosphate in Crayfish

In cooperation with scientists from the Ben-Gurion University in Israel we investigate the biomineralization of the freshwater crayfish *Cherax quadricarinatus*. The cuticle of this crustacean, which is regularly shed during every molting cycle, is reinforced with amorphous calcium carbonate. Surprisingly, investigations of the mandible of *C. quadricarinatus* showed that the molar extension, which is used for grinding, does not only consist of chitin and amorphous mineral, but also has a coating of crystalline apatite (see **Fig. 1**). This occurrence of apatite is very unusual for a crustacean. The structure and orientation of the apatite crystals found in the crayfish tooth are reminiscent of mammalian enamel. Moreover, the hardness profile of the crayfish molar, as measured by means of nanoindentation, was found to be remarkably similar to that of human teeth. Hence, the crayfish molar serves as an interesting example for the convergent evolution of a functional structure comparable to mammalian teeth. Furthermore, the crayfish mandibles as well as the crayfish gastroliths, which serve as calcium storage organs prior to molting, are used to study the effects of phosphate and different proteins for stabilizing amorphous calcium carbonate.

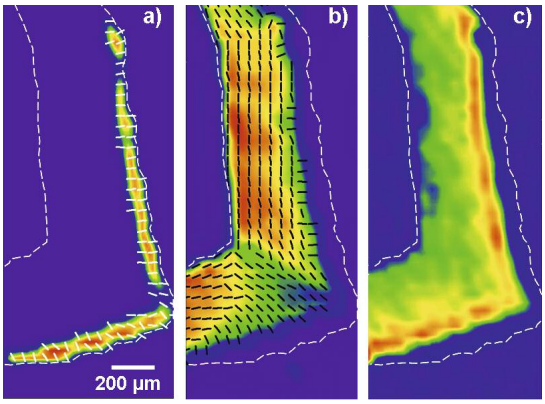

Fig. 1: Distribution of apatite (a), chitin (b) and amorphous mineral (c) in the molar of the crayfish Cherax quadricarinatus, evaluated from scanning WAXS measurements. The white and black lines in (a) and (b) indicate the orientation of the crystallographic c-axes of apatite and chitin, respectively. The dashed line follows the contour of the tooth cross section.

Biogenic Calcite Prisms from Mollusk Shells

Calcite from the outer layer of mollusk shells is known to contain intra-crystalline organic inclusions. We studied isolated calcite prisms from the shell of *Pinna nobilis* by means of combined synchrotron SAXS and WAXS **[3]**. A 3-dimensional reconstruction of the scattered intensity enabled us to relate the orientation of the nanostructure (SAXS signal arising from organic inclusions) to the crystallographic directions of the calcite lattice (WAXS). The results are shown as a stereographic projection of the integrated SAXS intensity (grey scale) together with the wide-angle spots of different calcite lattice planes as indicated by arrows (**Fig. 2**). A comparison of native and annealed calcite prisms, where the contrast for the latter is enhanced due to the removal of organics, shows that the organic-inorganic interfaces are preferentially oriented along the highly charged (001) lattice planes, most likely due to a strong interaction with negatively charged aspartate groups of the intra-crystalline proteins **[3]**.

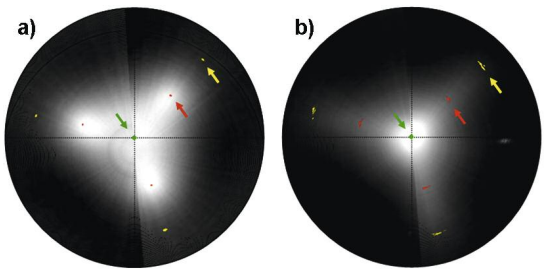

Fig. 2: Stereographic projection of SAXS intensity (grey scale) and wide-angle diffraction spots (green=(001), red=(104), yellow=(202)) for a native (a) and annealed (b) calcite prism isolated from a Pinna nobilis mollusk shell.

The observed (001) type interfaces help to explain the origin of previously reported lattice distortions in biogenic calcite, where the maximum distortion was found along the calcite c-axis. Moreover, additional studies on powder samples of prisms annealed at different temperatures gave proof that an initially rough organic-inorganic surface smoothens at 250° C **[3]**. This transition temperature coincides exactly with the one for the relaxation of the lattice parameters.

Bio-Inspired Calcite-Polyelectrolyte Particles

In order to find out whether we can observe similar effects as for the biogenic calcite, we investigate bio-inspired calcite particles with inclusions of polystyrenesulfonate (PSS). The work is carried out in cooperation with the Department of Colloid Chemistry. In spite of using only one soluble additive, we observed a highly complex hierarchical structure **[4]** (see **Fig. 3**).

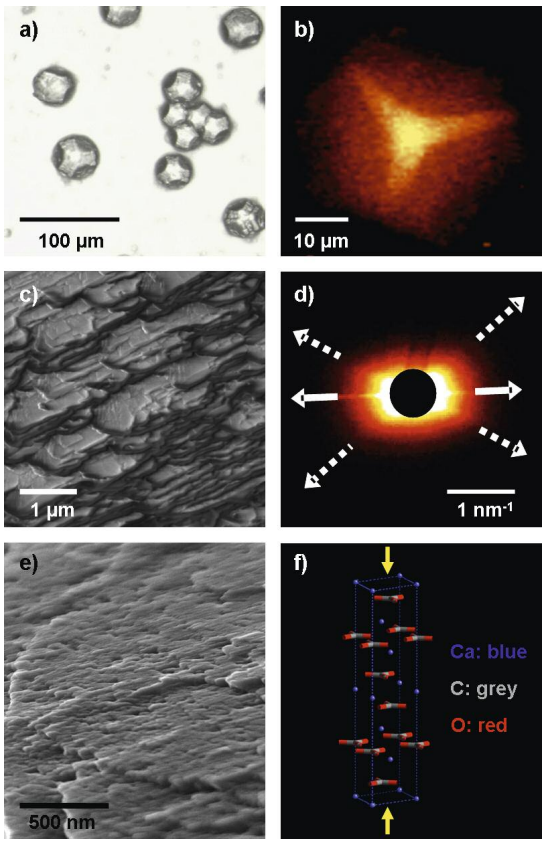

Fig. 3: Hierarchical structure of bio-inspired calcite-PSS hybrid particles: a) Light micrograph, b) Raman imaging of PSS (sulfonate group), c) SEM showing mineral building blocks, d) SAXS signal from interfaces with preferred orientations (dashed arrows: (104), full arrows: (001)), e) high resolution SEM showing a granular substructure, f) unit cell of the slightly distorted calcite lattice. The direction of the maximum contraction (c-axis) is indicated by yellow arrows.

The calcite-PSS hybrid particles, which are nucleated on the (001) plane, exhibit rounded edges (a) and variations in the polymer/mineral ratio on the µm-level (b). The intra-crystalline polyelectrolyte molecules induce a meso-scale arrangement of mineral building blocks in the 100 nm range (c), which show preferred orientations (d). Similar to biogenic calcite, the occurrence of organic-inorganic (001) interfaces in addition to the low energy (104) planes, arises from the interaction with negatively charged groups of the organic inclusions (aspartate in biogenic and sulfonate in biomimetic calcite). In addition, we observed a granular substructure of the mineral units (e) as well as a distortion of the calcite lattice structure (f), which is, however, much smaller than the effects previously found in biogenic calcite. The maximum distortion of the bio-inspired calcite, measured by means of high resolution X-ray diffraction at the European Synchrotron Radiation Facility in France, was a contraction of only 0.02% along the direction of the c-axes **[4]**.

Synchrotron Research at the µ-Spot Beamline

Together with different research groups of the MPI of Colloids and Interfaces, a wide range of materials including biological samples of bone, teeth, magnetotactic bacteria, cuticle and wood, as well as bio-inspired materials like the above mentioned polymer-mineral particles or cellulose based composites were investigated at the µ-Spot beamline. The spatially resolving scanning SAXS/WAXS and XRF (X-ray fluorescence) setup was for example successfully applied to investigate the effect of Strontium ranelate on the formation of human bone **[5]** and the formation of zebrafish fin bone via an amorphous precursor phase **[6]**. The performance of the experimental stage was continuously improved with respect to its resolution, variability of sample environments and the ability to characterize single crystals. This enabled us to study the structure of mercury-thiolate single crystals **[7]** as well as of organic inclusions in biogenic calcite **[3]**. We developed programs for a full 3D reconstruction of the reciprocal space from 2D measurements taken at different angles of rotation (results shown in **Fig. 2**). Furthermore, a method for the local chemical analysis of biomaterials by means of mapping lattice spacings was established **[8]**. A program for online data analysis is currently being developed in cooperation with DESY (Hamburg). The possibility to perform a fast data analysis is expected to have a major impact on future activities at the µ-Spot beamline.

B. Aichmayer, A. Al-Sawalmih, C. Gilow, W. Habraken, C. Li, T. H. Metzger, A. S. Schenk, B. Schonert, E. Schönemann, S. Siegel and I. Zlotnikov,
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References:

[1] Aichmayer, B., Fratzl, P.: Vielseitige Biominerale. Physik Journal **9**, 33 (2010).
[2] Aichmayer, B., Wiedemann-Bidlack, F. B., Gilow, C., Simmer, J. P., Yamakoshi, Y., Emmerling, F., Margolis, H. C.,Fratzl, P.: Amelogenin Nanoparticles in Suspension: Deviations from Spherical Shape and pH-Dependent Aggregation. Biomacromolecules **11**, 369 (2010).
[3] Gilow, C., Zolotoyabko, E., Paris, O., Fratzl, P., Aichmayer, B.: The nanostructure of biogenic calcite: a view by small-angle X-ray scattering. Submitted.
[4] Schenk, A. S.: On the Structure of Bio-Inspired Calcite-Polymer Hybrid Crystals. Doctoral Thesis, submitted to the University of Potsdam, Germany (2011).
[5] Li, C. H., Paris, O., Siegel, S., Roschger, P., Paschalis, E. P., Klaushofer, K., Fratzl, P.: Strontium Is Incorporated Into Mineral Crystals Only in Newly Formed Bone During Strontium Ranelate Treatment. J. Bone Mi. Res. **25**, 968 (2010).
[6] Mahamid, J., Aichmayer, B., Shimoni, E., Ziblat, R., Li, C. H., Siegel, S., Paris, O., Fratzl, P., Weiner, S., Addadi, L.: Mapping amorphous calcium phosphate transformation into crystalline mineral from the cell to the bone in zebrafish fin rays. Proc. Natl. Acad. Sci. USA **107**, 6316 (2010).
[7] Pokroy, B., Aichmayer, B., Schenk, A. S., Haimov, B., Kang, S. H., Fratzl, P., Aizenberg, J.: Sonication-Assisted Synthesis of Large, High-Quality Mercury Thiolate Single Crystals Directly from Liquid Mercury. J. Am. Chem. Soc. **132**, 14355 (2010).
[8] Paris, O., Aichmayer, B., Al-Sawalmih, A., Li, C. H., Siegel, S., Fratzl, P.: Mapping lattice spacing and composition in biological materials by means of microbeam X-ray diffraction. Adv. Eng. Mat., published online: DOI 10.1002/adem. 201000287 (2011).

Advanced Raman Spectroscopic Imaging of Biological Tissues

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Biological materials, such as wood or bone, are structured in a hierarchical way over many length scales. **[1]** Conventional single point Raman spectroscopy is inadequate to describe the chemical information and orientation distribution in relation to the macroscale. The focus of our research is *in-situ* studies of biological tissues at various hierarchical levels of structure (from molecular to tissue scale) taking advantage of advanced Raman imaging techniques. One of the goals of our work is to map collagen fibril orientation in tissues by evaluating the molecular response within the tissue to a polarized laser source (**Fig. 1**) **[2]**.

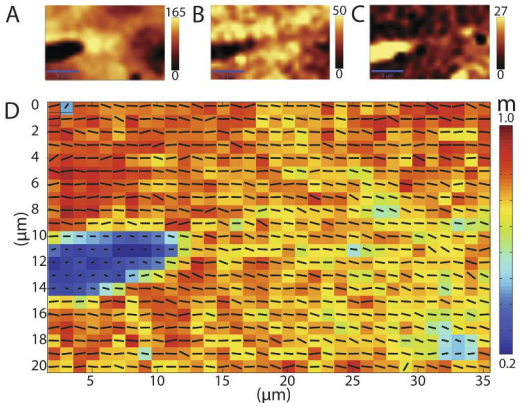

Fig. 1: Polarized Raman mapping of the collagen fibril orientation in mouse bone. The top images show chemical distribution of mineral (A), collagen (B) and embedding material (C) respectively. (D) Collagen orientation map obtained by fitting 13 Raman images collected with different polarization angles of the incident laser light. The direction of lines indicates the orientation of collagen fibrils, their length as well as the color code are related to the amount and three-dimensional organization of collagen molecules.

We are applying this methodology to investigate the evolution of collagen organization in hard and soft tissue formed in fracture gap (callus) during the process of fracture healing in rat bone (with J. Dunlop, Biomaterials, and G. Duda, Charité Hospital Berlin).

The main aim of our work is to link the structural organization and chemical composition to the physical properties of the biological material. One example is a collaboration with M. Harrington (Biomaterials) and J.H. Waite (University of California, Santa Barbara) where we used Raman spectroscopic imaging to study mussel byssal coating and plaque showing micron level spatial distribution of various proteins and their interaction with iron ions (**Fig. 2**) **[3, 4]**. One of the outcomes is that the unique mechanical performance of byssus coating is influenced by the specific distribution of protein-iron cross-links in locally concentrated areas.

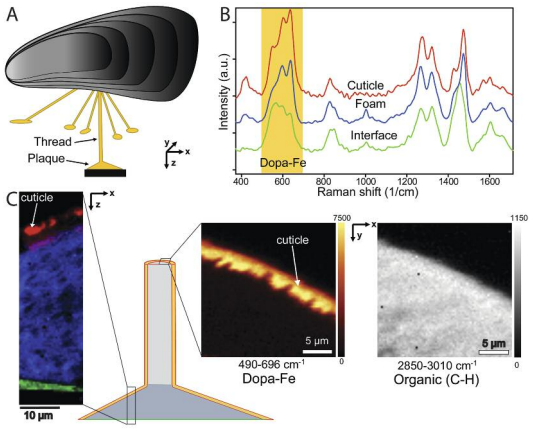

Fig. 2: Raman imaging of Mussel byssus thread and plaque. (A) Mussels produce a holdfast known as a byssus which is composed of extensible and shock absorbing byssal fibers. (B) Average Raman spectra of three morphologically distinct domains in byssus i.e. cuticle, foam, and plaque-substrate interface. (C) Raman images of plaque (left) and thread (right) cross-sections. In the plaque image the distribution of average spectra shown in B is visualized through least square fitting. In the thread images Raman band integrals of dopa-Fe (490-696 cm^{-1}) and overall organic content (2850-3010 cm^{-1}) are shown. Protein-Fe coordination cross-links in the thread result confined to the cuticle region.

Additionally, in collaboration with BAM (I. Rabin) and BESSY (U. Schade), we work on damage assessment of the Dead Sea Scrolls. Combining far infrared and polarized Raman spectroscopy we try to define markers related to changes in collagen molecules caused by deterioration **[5]**.

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References:
[1] Fratzl, P., Weinkamer, R.: Nature's hierarchical materials. Prog. Mater. Sci. **52**: 1263-1334 (2007).
[2] Gamsjaeger, S., Masic, A., Roschger, P., Kazanci, M., Dunlop, J.W.C., Klaushofer, K., Paschalis, E.P., Fratzl, P.: Cortical bone composition and orientation as a function of animal and tissue age in mice by Raman spectroscopy. Bone **47**: 392-399 (2010).
[3] Hwang, D.S., Zeng, H.B., Masic, A., Harrington, M.J., Israelachvili, J.N., Waite, J.H.: Protein- and Metal-dependent Interactions of a Prominent Protein in Mussel Adhesive Plaques. Journal of Biological Chemistry **285**: 25850-25858 (2010).
[4] Harrington, M.J., Masic, A., Holten-Andersen, N., Waite, J.H., Fratzl, P.: Iron-Clad Fibers: A Metal-Based Biological Strategy for Hard Flexible Coatings. Science **328**: 216-220 (2010).
[5] Rabin, I., Hahn, O., Wolff, T., Masic, A., Weinberg, G.: On the Origin of the Ink of the Thanksgiving Scroll (1QHodayota). Dead Sea Discoveries **16**: 97-106 (2009).

Nanometer and Micrometer Studies of Human Teeth and Relations to Dental Restorations

Human teeth do not re-grow, remodel or heal. Yet they function under cyclic mechanical load for many years in the harsh environment of the mouth. It may be hypothesized that the subtle variations observed in the microstructure of dentine and enamel – significantly contribute to the long term durability of human teeth. Understanding the details of the microstructure is therefore of great potential interest for new materials inspiration and design, and also for understanding failure in conventional dental treatment **[1]**. Our work centres on imaging wet human teeth by 2 dimensional (surface) and 3 dimensional (3D, volume) X-ray measurement methods: tomography, diffraction and small-angle scattering. We study the relations between the nanometre-sized carbonated apatite particles and the whole tooth structures (**Fig. 1**) **[2, 3]**. These findings we can now relate also to the 3D distributions of the dominant micrometer sized features of dentine, the dentinal tubules **[4]**. We have seen for instance that the mineral particles change their average orientations on the flanks of teeth and on the chewing surfaces, and that they also re-arrange from being randomly orientated to being more highly aligned in regions beneath the cusps. This suggests a design on the nanometre length-scale that matches the millimetre to centimetre length-scale structure and function of teeth.

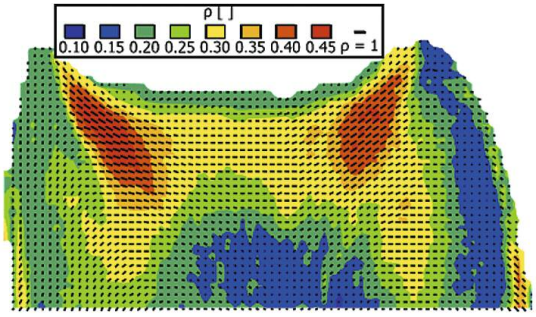

Fig 1. Collagen protein fibres in dentine of teeth are reinforced with tiny apatite particles. The orientation of these 2-4 nm thick particles, as seen by small angle X-ray scattering [2, 3] reveals an arrangement that appears to match and support load under the cusps (indicated in red in the upper left and right regions of the dentin silhouette. ρ represents the degree of co-alignment of the particles in a volume of about 50×50×150μm^3 and is zero for random distribution and is one for regions where particles are fully co-aligned).

Teeth have an important long-term mechanical functional role, and even if they are infected, they are often restored back into function. Imaging the microstructures of teeth and dentine are of particular potential use in benefitting clinical dentistry problems of matching fillings to the tooth microstructures. Indeed, the margins of fillings and restorations are key determinants for the quality of dental treatment, providing some measure of the ability of restorations to function for extended periods of time in the mouth **[1]**. However, it remains unclear to what extent leakage and bad fittings of restorations indicate failure and the need for new treatment. A growing availability of new phase-imaging methods allows us to investigate the interfaces between teeth and restorations **[5]** and to better observe and measure regions where the biomaterials should create durable interfaces with the natural tooth tissues (**Fig. 2**). These methods are also being used in basic studies such as the investigation of molar teeth of the crayfish Cherax quadricarinatus.

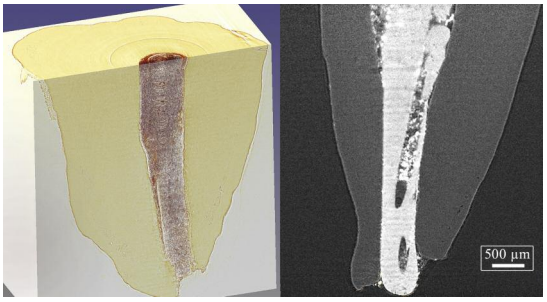

Fig 2. Root canal therapy with details revealed by phase-contrast enhanced micro tomography. A 3D representation (left) and 2D slice through the data (right), reveal what may be seen in a typical human root (root appearing in yellow) that is filled with dense conventional silver-containing filling/cement biomaterials (depicted in orange). The virtual data is vertically sliced so as to visually expose the internal structures. Although densely packed by an expert dentist, the filling biomaterials contain voids and inhomogeneities, with visible discontinuities near the dentine [5]. Gaps and unfilled regions spanning less than 20 micrometers can be seen in such fillings, and they have important but still unconfirmed clinical significance for long-term success.

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References:

[1] U. Blunck, P. Zaslansky: Enamel Margin Integrity of Class I One-bottle All-in-one Adhesives-based Restorations, doi:10.3290/j.jad.a18445, (2010)
[2] A. Märten, P. Fratzl, O. Paris, P. Zaslansky: On the mineral in collagen of human crown dentine. Biomaterials **31** (20):5479-90, (2010)
[3] A. Märten: Dreidimensionale Charakterisierung der Mikrostruktur des Dentins, Doctoral Thesis, Technische Universität Berlin (2010).
[4] P. Zaslansky, S. Zabler, P. Fratzl: 3D variations in human crown dentin tubule orientation: a phase-contrast microtomography study. Dent Mater **26** (1):e1-10 (2010)
[5] P. Zaslansky, P. Fratzl, A. Rack, M.-K. Wu, P. R. Wesselink and H. Shemesh: Identification of root filling interfaces by microscopy and tomography methods. Int. Endod. J **44**(5) 395-401 (2011)