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→ Biological Materials
→ Biological and Biomimetic Materials
→ Bio-Inspired Materials

BIOMATERIALS

Research in the Department of Biomaterials

The Department of Biomaterials conducts interdisciplinary research at the interface between materials science and biology. The approach is to elucidate the basic mechanisms by which the hierarchical structure of a variety of biological or bio-inspired materials leads to mechanical performance. The principle goals are:

(1) to provide new concepts for developing new materials inspired from nature,
(2) to contribute to the understanding of the biological tissue itself, for example in the context of biomedical problems.

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. The Department is organised 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 special methodology (such as synchrotron research or mathematical modelling). In this way, a expertise in a given field is maintained in each of the groups and strong scientific interaction and collaboration between them helps addressing scientific problems at the interface between various disciplines. Typically, these research groups comprise – in addition to the group leader – several doctoral students, postdocs, one or two technicians and responsibility for laboratories and heavy instrumentation maintained for the institute as a whole. In addition to the research groups, several independent postdoctoral researchers, some of them with individual grants from the Humboldt Foundation or other organisations, work on chosen scientific projects but without responsibility for a larger group.

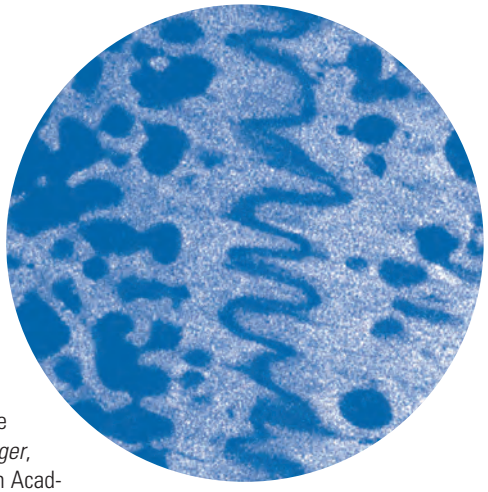
Generally, the experimental approach is based on multi-method imaging where different probes are used to image the same specimen. This combines different type of information, such as microstructure, chemical composition, mechanical properties in a position-resolved way with a resolution in the micron range. We are currently using scanning electron microscopy and scanning x-ray diffraction to characterize the micro- and nanostructure. We have established polarized and confocal Raman imaging to provide information on chemical composition and fibre orientation and we use nanoindentation 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. This helps finding structure-property relations even in extremely heterogeneous materials with hierarchical structure.

In a second type of approach, we study changes in a material (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 at the origin of the often outstanding properties of these materials. In some cases, this 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 has been set up over the last years at the synchrotron BESSY at the Helmholtz-Zentrum Berlin (see report by *O. Paris*).

The report from the Department of Biomaterials is structured in three sections, from biological to biomimetic research. Bone research is a major activity, addressing fundamental questions about the hierarchical structure of bone and its relation to mechanical performance as well as medical questions related to osteoporosis and to fracture healing. Bone is a tissue primarily composed of collagen, the most abundant protein in our body, and nanocrystals of carbonated hydroxyapatite, a calcium phosphate mineral. Fundamental questions about how bone deforms under external loads and how it hinders the propagation of cracks have been addressed during the last years in the research group led by *Himadri Gupta* on **hierarchical connective tissues** (p. 36). This work was extended to other collagenous tissues, such as the deer antler or tendon. A major achievement was the discovery of a shearing mechanism between mineralized collagen fibrils that protects fibrils from premature fracturing. *Himadri Gupta* moved at the end of 2008 to a lecturer position at Queen Mary University, London.

In addition, bone micro-structure is studied in the context of **bone material quality and osteoporosis** (p. 34) mostly in collaboration with the Ludwig Boltzmann Institute of Osteology (Vienna, Austria). The rationale behind these studies is that osteoporotic bone fractures, which have generally been associated with bone loss, may also be linked to (age- or disease-related) changes in the bone material itself. A wide portfolio of techniques has been established in the Department during the last years for the characterisation of bone biopsies from patients in clinical studies, for example. Currently, extensive work is done in establishing polarized Raman imaging for these purposes (see report by *Admir Mašić).*

An extensive collaboration in the field bone regeneration was established 2007 in the Berlin area by the SFB760 on musculoskeletal regeneration (financed by DFG) and the Berlin-Brandenburg School of Regenerative Medicine (a



graduate school funded in the framework of the German excellence initiative). These consortia are coordinated by the medical University Charité in Berlin and the Department of Biomaterials is actively involved with scientific projects as well as in the various steering boards. Scientific activities in the context of **bone regeneration** are reported by *Manjubala Inderchand* (p. 38) and include characterisation of structure and material properties of the fracture callus, as well as fundamental in-vitro studies of bone tissue growth on 3D scaffolds.

Theoretical modelling of bone formation, resorption, mineralisation and healing, as well as other research in the context of **mechanobiology** are reported by *Richard Weinkamer* (p. 40). A large fraction of this work is carried out in collaboration with the two consortia mentioned above (Ludwig-Boltzmann Institute, on the one hand, and the BSRT and SFB760 on the other). One of the highlights is the use of theoretical methods to extract information on the mineralisation kinetics from a bone mineral density distribution that can be measured with a single biopsy from a patient.

A second block of activities is summarized under the title of Biological and Biomimetic Materials. Structure and its relationship to mechanical function are investigated for a diversity of biological systems with the aim to extract principles as inspiration for the biomimetic design of new materials or systems. In the group led by *Ingo Burgert*, research on **plant biomechanics and biomimetics** (see p. 42) focuses on the plant wall, on its structure and properties and on developing ideas about how to generate new composites based on the design principles observed in plants. One of the interesting functions in this context is humidity-driven actuation. This plays an important role in plant actuation, in seed dispersal or in the generation of growth stresses.

Damien Faivre started in 2007 a research group working on magnetotactic bacteria containing magnetite nanoparticles for orientation in the earth's magnetic field. These particles are usually arranged in chains. Current research work on **molecular biomimetics and magnet biomineralization** (see p. 44) investigates possible differences between biogenic and artificial magnetite particles, as well as the role of proteins (in particular MamJ and MamK) for controlling the nucleation and growth of these particles and the formation of the chain structure.

Further research in biological and biomimetic materials is conducted in collaboration with external partners and by several independent postdoctoral researchers. The general topic is to understand the path **from micro-structure to mechanical function** (p. 46). *John Dunlop*, reports work on modelling tissue growth and plant movements, two topics with a close relationship to experiments conducted in the Department. *John Dunlop* has been Humboldt Fellow in the Department and is starting a new research group from the end of 2008. *Paul Zaslansky* describes his work, mostly based on x-ray and neutron tomography, to elucidate the relation between structural features in dentin, in enamel and at the dentin-enamel junction with the mechanical response of an entire tooth. Indeed, some of these structures may potentially be optimized for the tooth's function and should not be altered in restorations. *Notburga Gierlinger*, an APART fellow supported by the Austrian Academy of Sciences, is describing her work on the structure of the plant cell wall and on composites based on cellulose whiskers. In addition, theoretical work with external collaborators has brought new insights into the mechanical behaviour of layered and cellular materials which mimic biological materials such as glass sponges or cancellous bone. Some of this work is carried out with *Dieter Fischer*, Professor of Mechanics at the University of Leoben, who recently received a Humboldt Award to visit the Max Planck Institute of Colloids and Interfaces.

A last section summarizes the work of two research groups on bio-inspired materials. *Oskar Paris* reports on **mesoscale materials and synchrotron research** (p. 52). Interesting structures in cellulose- and chitin-based biological materials are revealed by micro-diffraction. The thermal transformation of such (mineral-loaded) biological materials generates ceramic phases. These transformations as well as condensation processes within silica mesopores are studied by in-situ diffraction techniques. Most of this research uses synchrotron radiation and, in particular, the possibilities of the µSpot beamline at the BESSY synchrotron (Helmholtz Centre Berlin) mentioned earlier. *Oskar Paris* has been directing the design and the construction of the end station at this beamline. With February 2009 he moved as full professor and chair of the Institute of Physics to the University of Leoben in Austria.

His responsibility for the operation of the µSpot beamline has been taken over by *Barbara Aichmayer* who is heading a group on **biogenic minerals and bio-inspired nanocomposites** since 2007. She reports (p. 50) on the self-assembly of proteins responsible for enamel formation and on the internal (nano)-structure of natural and artificial calcite crystals grown in the presence of polymers. The basic aims of her group are to elucidate how (occluded) polymers are controlling the growth and the properties of inorganic crystals.

Finally, it should be mentioned that almost all of the research in the Department of Biomaterials is based on collaborations, inside the Department, with other Departments in the Institute and with many outside partners around the world who all deserve our sincere gratitude for working with us in such a nice way.

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Bone Material Quality and Osteoporosis

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1998-2003: Chair of Metal Physics (University Leoben, Austria)
Director (Erich Schmid Institute for Materials Science of the Austrian Academy of Sciences)
Since 2003: Director, Department of Biomaterials (Max Planck Institute of Colloid and Interfaces, Potsdam)
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The fracture resistance of bone is a crucial issue in bone diseases such as osteoporosis and it depends on many levels of hierarchical structure of bone **(Fig. 1)**. Understanding the structural basis of bone material quality is, therefore, essential for the assessment of diseases such as osteoporosis, for a critical evaluation of current therapies and to aid in their more targeted development. Current research on bone quality in osteoporosis is carried out primarily in close collaboration with the Ludwig Boltzmann Institute of Osteology (Vienna, Austria).

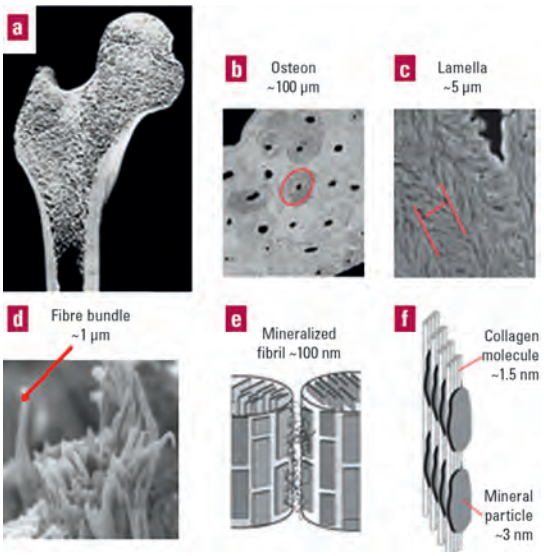

Fig. 1: Hierarchical structural levels in bone from the architecture of the human femoral head (a), osteonal structures surrounding blood vessels (b) via the lamellar (c) and fibrillar (d) organisations down to the nanoscale with mineralized fibrils (e) based on collagen and mineral nanoparticles (f). (from [1])

An ongoing activity is to assess the effect of osteoporosis treatments on bone material quality **[2]**. In recent years, the group has published a wide range of reference works, including an edited book **[3]**, several book chapters **[4-7]** and a review article **[8]**. In addition to the characterisation of the mineral distribution in bone tissue **(Fig. 2) [8]** and to the structural characteristics of the bone material at the nanoscale **[5, 6]**, particular attention has been paid to the validation of polarized Raman scattering for the characterization of collagen-based (mineralized) tissues **[7]**. The advantage of this technique is that it gives simultaneously information on the organic and on the inorganic component of bone. An analysis using a polarized laser beam gives additional information on local fibre orientations **[7]**. A more detailed description of these approaches is given on the next page by Admir Mašić, Postdoctoral Researcher supported by the Max Planck Research Award 2008 to PF.

Fig. 2: Bone trabecula from a biopsy visualized by back-scattered electron microscopy. Different grey scales indicate different mineral content. The local mineral content varies due to ongoing formation and resorption processes.

Bone can be present in a variety of forms fulfilling different mechanical functions. A first example is the deer antler, a particularly tough tissue (see Report of the group on mineralized tissues). A further example is the turtle shell which has been studied in collaboration with the group of Ron Shahar (Hebrew University, Israel). The shell of turtles is a shield which needs to be stiff at high loads but should provide sufficient flexibility for respiration and locomotion at smaller loads. We show that this seemingly contradictory requirement is met by a self-locking material, whereby stiff bony elements are connected by a much softer suture with a complex three-dimensional shape **(Fig. 3)**. A first description of this intricate tissue has just been published **[9]** (Highlighted as the Editor's Choice in Science 2009, 323: 438). Not only does this show a new level of organisation in bony tissue but this suture also shows an interesting principle of materials assembly with unusual mechanical behaviour.

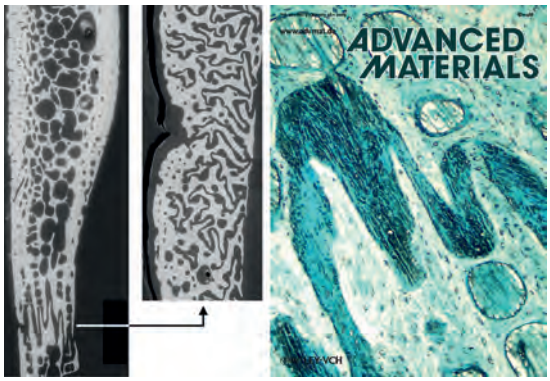

Fig. 3: Turtle shell consists of widened ribs (left) joined by an unmineralized suture (center) with a very complex shape. The central picture represents a cross-section through the suture region (arrow). The suture is filled with aligned organic fibres joining the bony parts (right). (from [9])

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Mapping Collagen-rich Tissue by Polarized Raman Spectroscopy

The collagen molecule is a fundamental structural building block for various types of natural tissues **[1]**. Its characteristic hierarchical structure, from atomic to tissue levels, allows for the fulfillment of a variety of mechanical functions, particularly in vertebrates. It is a major constituent of tendons and ligaments, as well as the organic matrix of bone and dentin – it is also present in skin and arteries. In all the aforementioned biological materials, the orientation of collagen fibers plays a fundamental role in the overall mechanical properties of the tissue. The significance of the collagen network and its architecture for normal physiological function can be witnessed when damage in one or both properties results in diseases such as osteoarthritis, skin cancer, osteogenesis imperfecta, etc. **[10]**

The aim of our work is to image collagen fibril orientation of tissues in situ by evaluating the molecular response within the tissue to a polarized laser source. For these purposes, we use Raman micro-spectroscopic and imaging analyses to elucidate collagen fibril orientation on micron scale.

Conventional single point Raman spectroscopy is inadequate to describe the chemical information and orientation distribution in relation to the macroscale. Recently, our group demonstrated the use of Raman imaging techniques in describing orientation and composition in cortical bone tissue **[7, 11]**.

In the present work we used polarized Raman micro-spectroscopy to obtain the diagonal, normalized components of the associated Raman tensor for the Amide I band in rat tail tendon (RTT). Obtained information was applied to process a series of Amide I Raman intensity images obtained with different orientation of incident laser polarization in Raman experiments. **Fig. 1** shows the map of the calculated orientation of the collagen fibers (direction of black lines). The length of the lines and the pixel color in the **Fig. 1** are related to the out of plane orientation of the collagen fibrils as well as the total amount of the Amide I band generating molecules. The calculated collagen orientation map is in good agreement with the fiber directions seen using optical microscopy **(Fig. 1A)**. The method can be applied to map collagen within other tissues, and in principal, it is possible to concurrently map other chemical components associated with collagen. The results demonstrate the versatility and potential of this analytical technique to image collagen fibril orientation within any tissue in-situ.

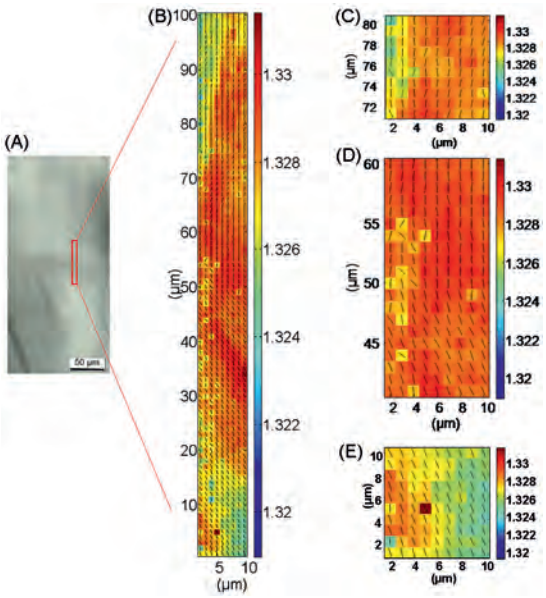

Fig. 1: In-situ polarized Raman mapping of the collagen fiber orientation in unstretched rat tail tendon (A) Optical microscopy image of the analyzed region where the crimp structure of collagen is visible (scale bar = 50 micron). (B) Map obtained by fitting 13 Raman images collected with different polarization angles of the incident laser light. The direction of arrows indicate the orientation of collagen fibers, their length represents the amplitude of the fitting curve, and the color code represents the average relative intensity of the Amide I band. (C), (D) and (E) Magnified regions of interest reveal specific structural changes in the tissue. Note the radical change in collagen fiber orientation corresponding to the crimp (at about 50 μm).

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Hierarchical Connective Tissues

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Biomineralized systems are hierarchically designed structures whose mechanical properties depend on multiple mechanisms at different length scales. They have a very high work of fracture, which is believed to arise from cooperative failure mechanisms at the nano- through micro level. We develop in-situ micromechanical and synchrotron-based methods to get answers as to how nature builds strong hierarchical systems. Our results find application in medical fields (for example to prevent bone fracture in osteoporosis) and in the design of new materials.

Nanoscale Fracture Mechanisms in Antler

Antler is a unique biomineralized organ in that it is annually regenerated completely and is used for a combat weapon during mating season by competing male deer. This makes it both an ideal model system for studying development of a biomineralized structure as well as an excellent example of a very high toughness structure tuned to function. Using in-situ synchrotron radiation combined with small-angle X-ray diffraction (SAXD), the (nanoscale) fibril strain was measured concurrently with macroscale tissue strain **[1]**. We observed a dramatic increase in SAXD peak width after mechanical yielding, indicative of decoupling between fibrils and heterogeneous fibrillar deformation. This result led us to a nanoscale model for the high toughness of antler, as shown in **Fig. 1**.

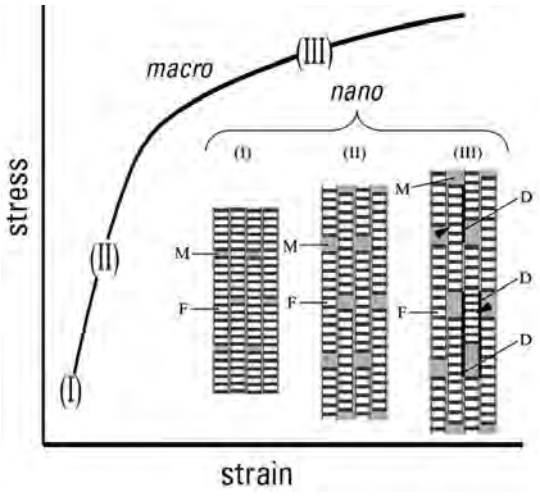

Fig. 1: Nanoscale model of heterogeneous fibrillar elongation in antler in the post yield(II – III) inelastic zone during macroscopic tensile deformation

High Microscale Mechanical Anisotropy of Bone

A crucial structural feature of bone (at multiple length scales) is the high structural anisotropy, with long mineralized collagen fibrils at the nanoscale assembling in twisted plywood lamellae at the micron level, which form cylindrical laminated structures (osteons) at the tissue level. In order to measure the mechanical anisotropy of the mineralized fibrils as far as possible, we considered individual structural components (bone packets) in the bovine bone periosteum. Using UV laser microdissection to cut out individual packets and thus avoid the complications of higher levels of hierarchy, microtensile tests were carried out on packets sectioned at different angles to the principle fiber axis. Our results reveal a very high mechanical anisotropy (100 to 1) in tensile strength and elastic modulus of these packets **(Fig. 2) [2]**.

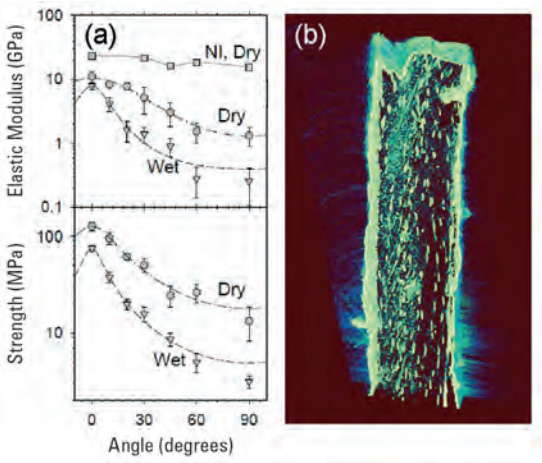

Fig. 2: High mechanical anisotropy of fibrolamellar bone packets as a function of angle to main fiber direction. A 3D X-ray microtomographic image of a bone packet is shown on the right.

Inelastic Deformation Banding in Bone

Little is known about the microscale processes operative during inelastic bone deformation, although these are expected to be quite different from those operating in simpler materials like alloys, polymers or ceramics. We developed a digital image correlation algorithm to measure the tissue strain distribution at the microscale (~100 µm) in bone **[3]**. Our result show that the elastic/inelastic transition is precisely the point, where, locally, one or more high deformation bands appear across the tissue, and eventual fracture occurs in these high-deformation regions **(Fig. 3)**. These results both provide important information on the microscale toughening mechanism as well as call into question use of simple parameters like ultimate fracture strain to describe fracture in bone.

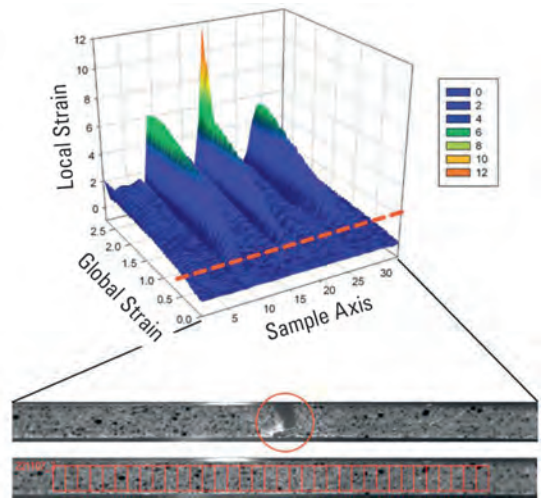

Fig. 3: High deformation banding occurring during inelastic deformation of bone. The lower two images show light-microscope images of bone-tissue and the tracking grid overlaid on the images to measure strain. The upper plot shows the local strain profile (vertical scale) along the sample axis as a function of global strain; 3 high-deformation bands are observed

Self Healing in the Connective Fibers of a Mussel

Some natural connective tissues exhibit remarkable mechanical and structural self-healing properties, and understanding the supramolecular origins of these qualities may help in designing synthetic self healing materials. The byssal threads of marine mussels are used as anchoring lines to secure the organism to the rock-bed in a wave swept seashore environment. While exhibiting elastic behavior at low strains (<15%), they can extend up to 100% strain without breaking, giving them properties comparable to Kevlar. They exhibit an acellular mechanical self-healing behavior over time after being stretched into the inelastic zone.

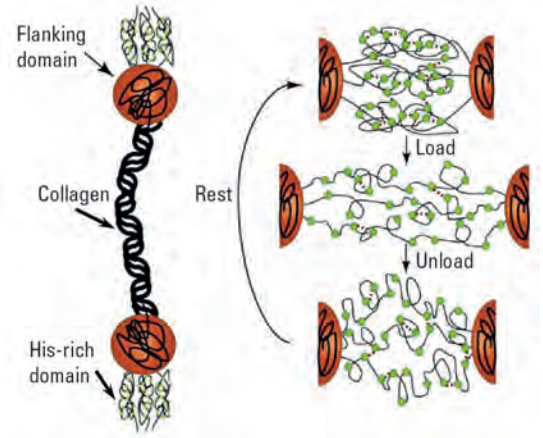

Fig. 4: Left: Molecular structure of the byssal fiber, indicating collagen domains, adjacent flanking domains and terminal histidine rich domains. Right: A molecular schematic of His-dependent healing in threads, by reformation of crosslinks.

We used synchrotron wide-angle X-ray diffraction with in-situ tensile testing to understand the molecular origins of this phenomenon. We find that the collagenous segment never exceeded strains of 2% despite the whole fiber exceeding over 70 % strain. This indicates a ductile non-collagenous component is crucial for the inelastic behavior. We propose that the histidine (His)-rich domains adjacent to the collagenous segment contain metal-His bonds, which are broken during inelastic loading and are eventually reformed during self healing **(Fig. 4)**. This suggest that by inserting molecular domains with such "sacrificial bonds" in series with stiff collagen segments, byssal fibers transform tendon-like fibers into much tougher and stretchable fibers with intrinsic self-healing capability **[4]**.

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[1] 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, in press (2009).

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Bone Regeneration

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Bone regeneration is influenced by biochemical, biomechanical as well as cellular mechanisms. Our general aim is to understand the fundamentals of underlying mechanism of new bone formation under different conditions such as in-vitro cell culture systems in scaffolds and in-vivo models of bone growth and development, and fracture healing conditions. Under in-vitro experiments, a biomimetic scaffolds with controlled architecture and varying pore size and shapes are used as substrate to investigate the kinetics of three dimensional growth of tissue produced by bone forming cells. Under in-vivo conditions, the new bone formation via fracture callus during bone healing process is studied, since little is known about the material properties of various types of tissues comprising the callus. Here we investigate the spatial and temporal sequential distribution of ultrastructure and mechanical properties of callus tissues. Both the projects have started in 2007 within the framework of Sonderforschungbereich (SFB) 760 focussed in Berlin with research partners from Charité-Universitätsmedizin Berlin and GKSS Institute for Polymer Research at Teltow. Although in medical terms the bone development from embryonal to mature bone is understood, the process of mineralisation and growth is not well known.

Bone Healing and Regeneration

After bone fracture, various cellular activities lead to the formation of different tissue types, which form the basis for the process of secondary bone healing. While the histological evaluations describe the spatial and temporal distribution of the various tissue types comprising the callus **(Fig. 1a)**, little is known of their material properties at various hierarchical level. We investigate the spatial distribution and temporal sequence of ultrastructure and mechanical properties of callus tissues over the course of bone healing by applying our established multi-method approach, whereby the same specimen is scanned to map tissue composition, mineral particle size and concentration, as well as mechanical properties at the local level with micrometer resolution, using scanning small- and wide-angle x-ray scattering, scanning electron microscopy, nanoindentation and acoustic microscopy.

This project is in close conjunction with the researchers at Charité-Universitätsmedizin Berlin, (G. Duda, CMSC) where the bone healing experiments is carried out both in small and large animal models, as it is known that the tissue architecture is quite different in different animal species. In one of the fracture healing model in sheep bone, it has been shown that the indentation modulus (elastic modulus) maps in selected regions of callus are heterogeneous and follow the architecture of the trabeculae in the mineralized callus **(Fig. 1b)** and the average modulus value after 9 weeks of healing (end point) appears to be half of that of normal bone **[1]**.

This experimental result paved way to correct the wrong assumption used in theoretical modeling where in the modulus value of mineralized callus is assumed to be equal to bone. The spatial and temporal distribution of mineral content in the callus tissue, measured by quantitative back scattered electron imaging, also illustrates the ongoing bone formation and remodelling process. The structural investigations predicts the growth of mineral particles during healing process in callus tissue while there is a decrease in mineral crystal characteristics in cortex at the fracture gap, indicating dissolution of mineral from bone at fracture gaps **[2]**.

Furthermore, understanding the bone healing process not only in the native state, but also under the influence and intervention of biological factors or physical stimuli on callus tissue formation, is necessary to evaluate the clinical conditions of fracture healing. Other animal models will be investigated in this context.

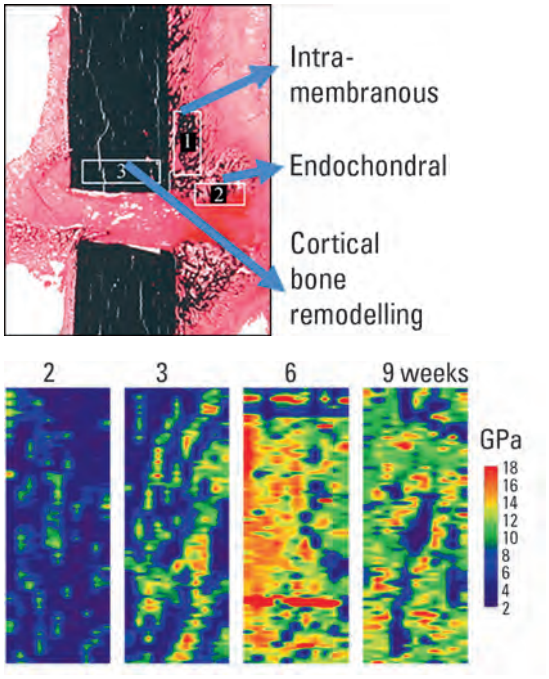

Fig. 1(a): The various tissues formed during fracture healing identified by histology (b) Indentation modulus maps of the intramembranous callus (region 1) over the course of healing in sheep fracture model.

Bone regeneration and remodelling around an implant is also studied in case of stainless steel and titanium nail implants using similar methodologies.

Bone Growth and Development

The knowledge how the mineral crystals in bone organise, nucleate and grow from the "birth of bone" (embryonal) is still poor. The study which aims to understand the development of the mineral properties, the mineral deposition and organisation from embryonal to mature bone has been now a part of graduate school in Berlin (cooperation with S. Mundlos, MPIMG). As it is already known that the genetic changes influence the material properties (mechanical properties) of bone **[3]**, the effect in embryonal bone level is not known and this will be studied further.

Bone Material Quality Related to Diseases and their Treatment

The changes occurring in bone material quality with respect to disease and their treatment is studied in close collaboration with the researchers at Ludwig Boltzmann Institute of Osteology in Vienna, Austria. The project deals with understanding the correlation of nano mechanical and nano-structural properties of diseased bone in relation to mineral content and treatment parameters in significant bone diseases such as osteoporosis and osteolathyrism.

Tissue Growth on Biomaterials of Controlled Geometry and Stiffness

Biomimetic scaffolds of controlled architecture are produced via solid freeform fabrication or rapid prototyping (RP) technique in which complex three dimensional (3D) structures can be produced directly from computer generated (CAD) design. The microstructure of the RP fabricated hydroxyapatite-chitosan/PLLA scaffolds were controlled by freeze drying process. The pre-osteoblastic cells cultured on scaffolds proliferated over the material and pores in multilayer and produced extra-cellular matrix in three weeks in both hydroxyapatite and polymer based composite scaffolds **(Fig. 2) [4, 5]**. The structure of the cell cultured scaffold allows designing the biomimetic scaffold with polymeric network inside the pores and enhances more cells to produce tissue compared to two-dimensional matrices **[6]**.

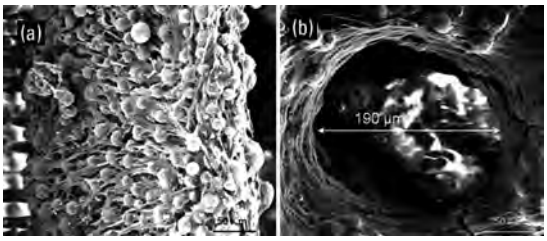

Fig. 2: SEM images of the cell cultured scaffold (a) showing the proliferated cells on one of the struts of scaffold and (b) cells filling up the pore channel with tissue and forming round canal.

The physical properties of scaffolds/substrates have a direct impact on cell proliferation and furthermore, on tissue formation. For this purpose a model system was established, which allowed in parallel microscopic observation as well as quantification of new tissue formation in a three-dimensional environment. The influence of various shapes and size of the pores in the hydroxyapatite scaffolds was studied and the tissue formation occurs in a way that is independent form the original shape, the tissue grows in round central canal form as observed with confocal laser scanning microscopy **(Fig. 3a) [7]**. The kinetics of tissue formation over of period of six weeks showed no shape dependence of the amount of tissue area, but revealed strong size dependence **(Fig. 3b)**.

Based on this information, various polymers with varying physical properties, especially, stiffness, are to be studied to analyse the effect on kinetics of tissue formation. Scaffolds from a series of polymer (polyurethane) with varying stiffness having various pore shapes and sizes was investigated to study the influence of the substrate stiffness on new bone tissue formation **[8]**. The kinetics study revealed that there are two stages of tissue growth compared to the stiffer hydroxyapatite material. The first early stage is dependent on substrate property and the second late stage is independent of the material **(Fig. 3c)**. Further studies will be based on other polymers with stiffness varying from kPa to MPa range that will be developed by our collaborating partners (A. Lendlein) from GKSS Institute for polymer research.

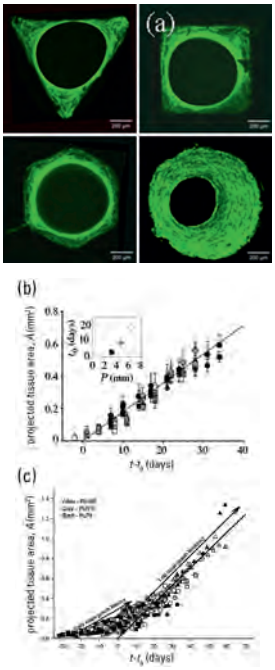

Fig. 3: (a) Extracellular matrix (ECM) tissue growth in 3D channels of various shapes in hydroxyapatite forming a round central channel, (b) showing that the growth is independent of shape and (c) tissue growth kinetics shows two stages in polymer scaffold.

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Mechanobiology

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Dynamical processes in bone are of great interest from both a materials and medical point of view. Computational models that take into account bone's structural hierarchy were employed to study the processes of mineralization, remodeling and fracture healing in bone. The second two processes are classic examples of mechanobiology **[1]**, in which cell action is mechanically controlled, the first being one which results in materials changes and thus mechanical changes within bone.

Bone Material Heterogeneity

On the microscopic length scale bone material quality is affected not only by the mean mineral content of the matrix, but also by the heterogeneity of the mineral content together with its spatial distribution. This heterogeneity of the mineralization results from the continuous remodeling, where a small bone volume is resorbed and replaced by an unmineralized bone packet. After the deposition, the mineralization process leads to an increase in the mineral content in the bone packet described by the mineralization law. The heterogeneous mineralization of trabecular bone is characterized by a frequency distribution, the bone mineralization density distribution (BMDD). We developed a mathematical model which relates the BMDD to the mineralization law **[2]**. Starting from the experimentally obtained BMDD of healthy human adults, the corresponding mineralization law was obtained. The investigation of a patient with a tumor-induced osteomalacia revealed profoundly disturbed mineralization kinetics **[3]**. The model was further applied to predict the full time evolution of the BMDD for two important clinical scenarios: menopause in women and anti-resorptive therapy. The simulations of increased bone turnover (menopause) resulted in a shift of the BMDD toward lower values of the mineral content with a significant transient broadening of the BMDD. Conversely, a decreased turnover (anti-resorptive therapy), caused the BMDD to shift towards higher values of the mineral content displaying a transient narrowing **[4]**. Additionally the model predicts the time evolution of the bone mineral density (BMD), which is used usually in the diagnosis of osteoporosis. The simulation showed that the strong reduction of the BMD after onset of menopause is only about half due to a loss in bone volume, whereas the other half is due to a reduction of the mineral content of bone **[4] (Fig. 1)**.

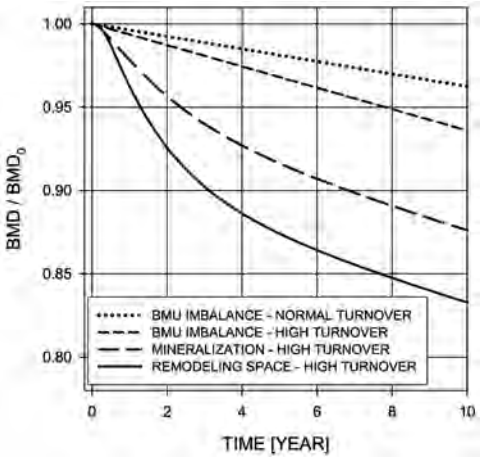

Fig. 1: Time evolution of the bone mineral density (BMD) after an increase in bone turnover simulating the onset of menopause (full line). An important contribution stems from the decrease in the mineral content, which is given in plot as the difference of the long and short dashed curves.

Adaptation of Trabecular Bone Architecture

On the mesoscopic length scale bone (re)modeling allows for the functional adaptation of the network-like architecture in trabecular bone to changes in the external loading. Consequently, the habitual loads on the bone should be reflected in its trabecular architecture. Together with anthropologists of the Max Planck Institute in Leipzig we used high resolution computed tomography and advanced image analysis techniques to analyze position resolved architecture in proximal femora of primates with different locomotor behaviors. The primates species analyzed were categorized as predominantly walkers, springers, brachiators or climbers. A local analysis was performed by moving a cubic volume of interest (VOI) of size $(5\,mm)^3$ throughout the proximal femur **[5]**. The obtained standard morphometric parameters like bone volume fraction (BV/TV), trabecular thickness (Tb.Th) and trabecular number (Tb.N) revealed two different mechanisms of trabecular bone adaptation **(Fig. 2)**. In highly loaded regions of the proximal femur, BV/TV increases by increasing the thickness of the trabeculae, while Tb.N remains constant. In less loaded regions, BV/TV decreases by reducing the number of the trabeculae while Tb.Th does not change. This reduction in Tb.N goes along with an increase in the degree of anisotropy, indicating an adaptive selection of trabeculae. The main orientation of the trabeculae in the femoral head is directed towards the femoral neck. Only the brachiator displays significantly lower trabecular anisotropy and a more radial arrangement within the femoral head.

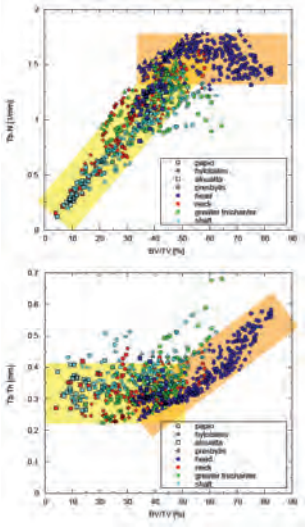

Fig. 2: Relation between the local bone volume fraction, BV/TV, and local trabecular number, Tb.N, and local trabecular thickness, Tb.Th, respectively. Data from all different primates and all different anatomical regions of the proximal femora are included (see figure legend). The primates differ in their locomotor behavior: papio (walker), hylobates (brachiator), alouatta (climber) and presbytis (springer).

Bone remodeling is thought to be mechanically controlled so that bone is removed locally where it is not mechanically needed and preferentially deposited at sites of high load (Wolff-Roux law). Using a computer model based on this mechanical control rule **[6]**, the best agreement between experimental data and simulation results were obtained, when a threshold for the local mechanical stimulus was assumed, above which strong bone deposition is activated **[7]**. In addition, we developed a stochastic model, which allows the extraction of information about the control of bone remodeling from measured trabecular thickness distributions (TTDs). In this Markov model each trabecula in a human vertebra is described by its thickness. Events of bone deposition or resorption change this thickness. Taking the TTD of young vertebrae as model input, a set of plausible remodeling rules for bone deposition/resorption could be obtained **(Fig. 3)**. These remodeling rules can then be used to predict the structural changes as described in the TTD as a function of age.

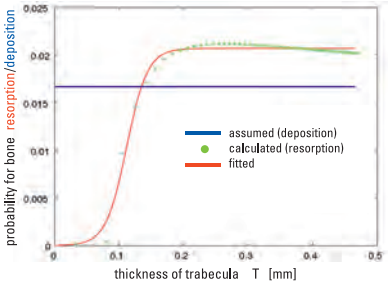

Fig. 3: Set of remodeling rules for the mechanical control of bone remodeling obtained on the basis of experimental data of the trabecular thickness distribution (TTD) of healthy bone. One remodeling rule has to be assumed the other can then be calculated.

Bone Fracture Healing

On the macroscopic length scale bone has the fascinating property to regenerate itself after a fracture, thereby returning basically to the prefracture state. Healing proceeds via a stabilisation of the bone fragments by the formation of an external callus and a succession of intricate patterns of different tissue types within this callus. Cell differentiation and the production of the different tissue types depend crucially on the local mechanical loading conditions **[8]**. We developed a computer model based on mechanobiological cell differentiation rules to be able to predict the course of healing in different scenarios. Beforehand we performed an analysis of healing data obtained from sheep to obtain quantitative data for comparison with simulations. An animal study of fracture healing within sheep was performed by our collaboration partners at the Charité, Berlin. The healing process in the tibia was monitored by means of longitudinal histological sections at 2, 3, 6 and 9 weeks postoperatively. The assembling of these histological sections to a succession of images that show the course of normal bone healing is significantly hampered by individual differences between the sheep. **Fig. 4** shows from the final result three from six obtained images displaying different stages in the healing process: the formation of new bone at the outer periosteal side, the development of cartilage within the fracture gap, the formation of a bony bridge at the outer side of the callus leading finally to a complete ossification.

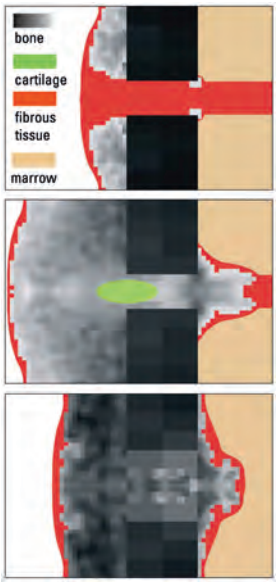

Fig. 4: Three different stages in the healing process of a long bone in sheep. The longitudinal sections through the cylindrical bone filled with marrow (only the left side is displayed) show the two bone fragments (black). Healing occurs by formation of a callus and an intricate temporal and spatial pattern of different tissue types.

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Plant Biomechanics and Biomimetics

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The research group Plant Biomechanics and Biomimetics investigates structure-function-relationships of plants at the micro- and nanoscale. Plant biomechanics provides a powerful tool to gather insights into the relationship of plant form and function as an expression of plant strategy to survive under given environmental conditions and physical constraints and is also a valuable source for extracting biomimetic principles.

Cell wall properties and plant actuation systems are analyzed to better understand the underlying principles and to utilize the gained knowledge for the design of innovative biomimetic materials.

Cell Wall Structure and Function

Plant cell walls consist of just a few nanometer thick cellulose fibrils as well as a matrix of hemicelluloses, pectins, lignin, and structural proteins. Their mechanical performance is based on the mechanical properties of the individual components and their interaction according to the polymer assembly. Consequently, the mechanical relevance of a cell wall component depends decisively on its distribution, spatial orientation, and bonding characteristics.

Our objective is to characterize this nanocomposite, in order to gain better insights into optimization strategies of living plants as well as into the material design as such. For this purpose we investigate primary cell walls of Arabidopsis hypocotyls and secondary cell walls mainly from spruce and aspen both in natural condition as well as genetically, chemically and enzymatically modified. The methods utilized are microtensile tests combined with X-ray scattering, Raman spectroscopy, FT-IR microscopy and Environmental Scanning Electron microscopy. Collaborations have been established in the framework of the EU-Project CASPIC as well as with the MPI for Molecular Plant Physiology MPI-MP), Potsdam.

In terms of primary cell walls of Arabidopsis we have continued and intensified our collaborations with plant physiologists, biochemists and biotechnologists to draw synergisms from the unique combination of enzymeology and genetic engineering on one hand and micromechanical characterization on the other hand.

In collaboration with the Markus Pauly Lab (now Michigan State University) we work on hemicelluloses in primary cell walls, mainly xyloglucan which is believed to build a load-bearing network together with the cellulose fibrils. Micromechanical analysis was contributed to a study on *Arabidopsis thaliana* deficient in xyloglucan in the primary cell walls due to the disruption of two *xylosyltransferase* genes. The obtained results challenge the common cell wall models **[1]**.

A further focus in primary cell wall research is on cellulose fibril orientation and its control by the plant. In collaboration with Staffan Persson from the MPI-MP we work on Arabidopsis plants which possess alterations in the cytoskeleton or in the cellulose synthase complexes due to I) chemical treatments and II) genetic modifications. In the framework of EU project CASPIC we work on transgene Arabidopsis plants with alterations in the protein structure of the cellulose synthase complexes (cesA2, cesA5, cesA6, cesA2/5 and cesA2/6) provided by the Lab of Herman Höfte (INRA-Versailles).

In terms of secondary cell walls further in-situ techniques have been established which combine micromechanical straining with nano- and microstructural observation. One achievement was a microtensile tester coupled with a cooling stage which allows mechanical tests of biomaterials in a fully hydrated state in a chamber of an Environmental Scanning Electron Microscope (ESEM), **(Fig. 1)**.

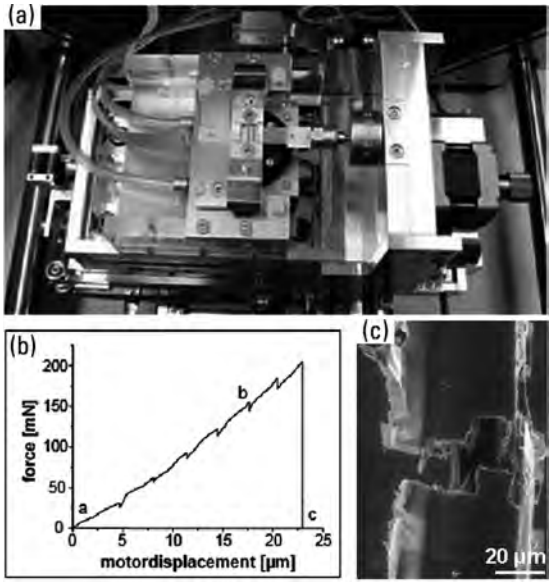

Fig. 1 (a) Tensile tester to be operated in the ESEM chamber; (b) Force-displacement curve of a single wood fibre (small load drops appeared when images were taken); (c) Single wood fibre after fracture [2].

In the framework of the EU project CASPIC transgene aspen plants with alterations in cellulose and lignin composition which had been provided by the Plant Science Center in Umea, Sweden (Lab Björn Sundberg) are investigated with respect to cell wall nanostructure and mechanical performance. Further genetically modified plants will be studied I) to learn about the control of cellulose fibril orientation in secondary cell walls and II) to better understand the cellulose fibril/matrix interactions in the cell wall assembly.

Exemplary studies on secondary cell wall led to a better understanding of structural and mechanical adaptations across growth rings in living trees **[3]** as well as to new insights into the cell wall nanostructure of softwood by means of cellulose fibril organisation **[4]**.

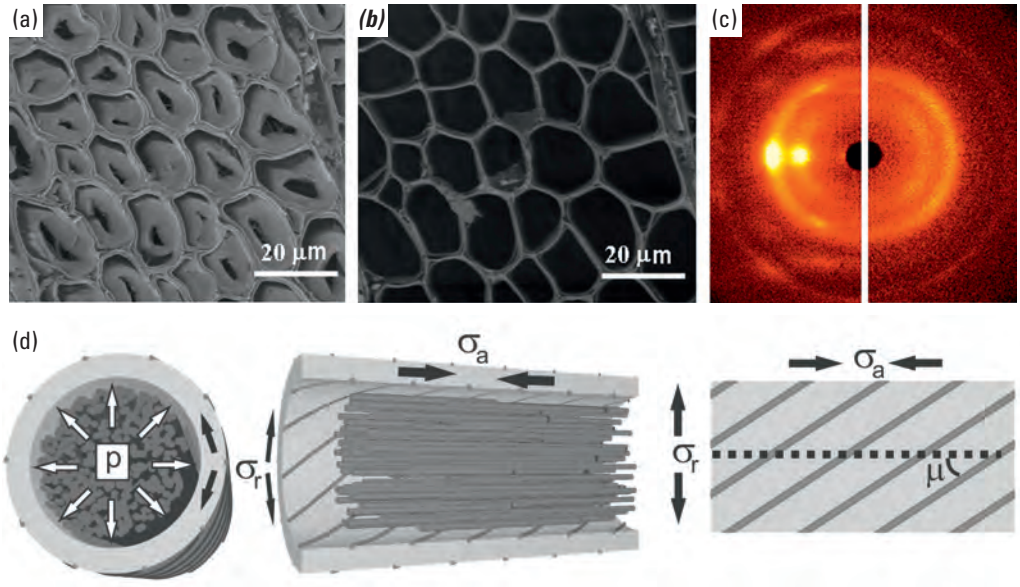

Fig. 2: (a) Effect of enzymatic treatment on the tension wood fibres. Scanning electron microscopy image of a a) cross-section of the native tension wood tissue with cell lumina almost completely filled with G-layers; (b) SEM image of the same tissue after enzymatic treatment with complete degradation of the G-layers; (c) WAXS diffraction pattern of poplar tension wood with G-layers (left) and after enzymatic removal of the G-layers (right). (d) Schematic drawing of the stress generation mechanism. The pressure p generated by the swelling of the G-layer is transferred into a circumferential hoop stress σ_r within the cell wall which is converted into an axial tensile stress σ_a [7].

Stress Generation and Plant Movement

Investigations on directed movements of plants at long time scales (wheat awns, reaction wood of trees) which do not require any metabolism but are triggered simply by the swelling or shrinking of the cell walls have been conducted in close cooperation with Peter Fratzl **[5]**, **[6]**.

In a recent study we examined the underlying principle of stress generation in tension wood of poplar **(Fig. 2)**.

Tension wood enables hardwoods to generate very high tensile stresses on the upper side of a bending organ such as to pull leaning stems and branches upwards. The tension wood fibres tend to contract longitudinally during differentiation which generates high longitudinal tensile stresses. A gelatinous layer (G-layer) filling the lumen of the fibre is believed to be the operative part of the tension wood fibre. The fundamental question is how the length of tension wood fibres can be reduced by a G-layer consisting of axially oriented almost non contractile cellulose fibrils. This can be explained by an interaction with the spiral arrangement of cellulose fibrils in the secondary cell wall, by which the circumferential hoop stress is converted into a contraction of the cell along its length. It has been shown in a mechanical model that the optimal spiral angle for the generation of longitudinal contractile stresses is close to the observed microfibril angle of ~36°. Hence, the combination of an axially stiff and laterally swellable G-layer with a suitable cellulose microfibril angle in the secondary cell wall is responsible for the generation of considerable high tensile stresses in poplar.

Bio-inspired Materials

In the field of biomimetic research we finalized the work carried out in cooperation with the University of Freiburg (Lab Thomas Speck) on gradient transitions in arborescent palms with *Washingtonia robusta* as a model organism. It has been shown that a stiffness gradient is accommodated by the specific cell and cell wall structure of the stiff vascular fibres bundles which helps to avoid critical stress discontinuities and separation of the material at the interface to the soft parenchymatous tissues **[8]**.

Two projects on synthetic systems which are inspired by the fibre composite structure of plant cell walls are ongoing. Together with colleagues from the Department of Interfaces (Labs Dayang Wang, Rumen Krastev) we produce and characterize hydrogels which should become anisotropic and switchable due to the embedding of fibrillar components (DFG project).

In cooperation with colleagues from the Department of Colloids (Lab Helmut Schlaad), partners from the University of Bayreuth (Lab Andreas Fery), the University of Freiburg (Lab Thomas Speck), and from the ITV Denkendorf (Lab Markus Milwich) we develop innovative glass fibre composites in the framework of a BMBF project. Here the design of the interface between glass fibre and resin matrix is inspired by the embedding of cellulose microfibrils in the plant cell wall.

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Molecular Biomimetics and Magnet Biomineralization

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The formation of inorganic materials with complex form is a widespread biological phenomenon (biomineralization) that occurs in almost all taxonomic groups from prokaryotes to humans. Spectacular examples of biomineralization are found in magnetotactic bacteria that not only synthesized magnetite or greigite nanoparticles with a great variety of morphology within dedicated organelles called magnetosomes **(Fig. 1)**, but also arrange them in one or more chains in order to create an ensemble with enhanced magnetic properties **(Fig. 2) [1]**. These complex structures made of assembled biomineralized magnetic nanoparticles reveal our limited understanding of a fundamental question: How does a cell translate DNA sequence information into patterned three-dimensional organization?

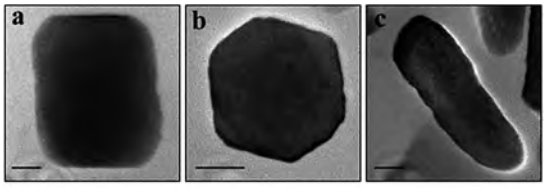

Fig. 1: Possible morphologies observed for magnetosomes based on high-resolution TEM images: A parallelepipedal projection of a possibly peudo-hexagonal prismatic morphology,B hexagonal projection of a possibly cuboctahedral crystal and C tooth-shaped (anisotropic) magnetosomes (the scale bar represents 20 nm).

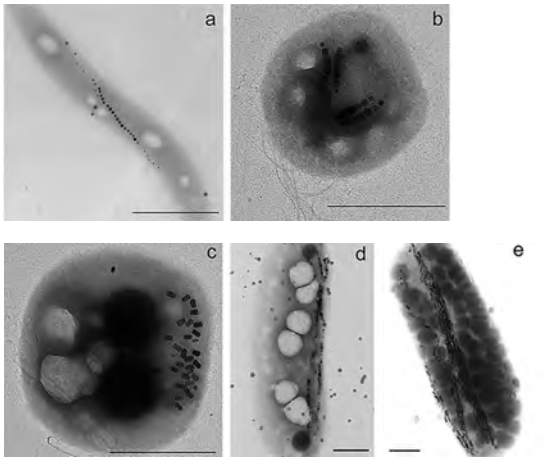

Fig. 2: TEM images showing the diversity of morphologies of magnetotactic bacteria and of the arrangement of magnetosomes (scale bar 1 µm). Morphologies include spirilla (a), cocci (b and c), rod-shaped (d) and vibrio-shaped microorganisms. Magnetosomes can be arranged in one (a) or several chains (b, d and e), or formed clusters (c).

Magnetotactic bacteria have thus mastered the combination of two contradictory capabilities: the biosynthesis of complex structures with high fidelity, and the seemingly infinite variation of this process. Consequently, magnetosomes are typically the result of highly efficient but complex natural processes that provide an ideal basis for developing biomimetic concepts towards new classes of magnetic components based on nanoparticles and their assembly.

Biological Materials

We, first, developed a technique that enables the study of magnetosome formation and assembly independently of cell growth **[2, 3]**. In the last months, the crystal structure of magnetosomes was studied by wide angle X-ray scattering with Synchrotron radiation in order to obtain information about a possible difference between biogenic and abiotic (synthetic) magnetite. Our first results indicate a reduced but clear isotropic lattice distortion of the magnetosomes relative to the inorganic magnetite control **(Fig. 3)**. Moreover, opposed peakshifts were observed in biogenic vs. abiogenic magnetite through annealing at 400 °C under inert atmosphere **(Fig. 3)**.

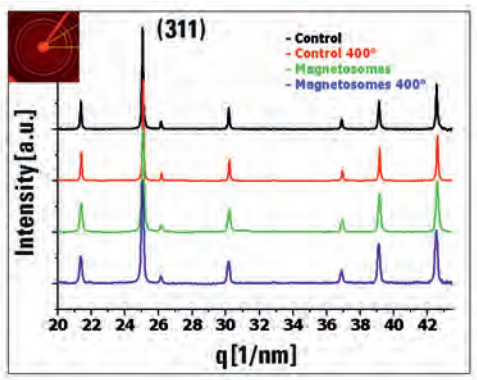

Fig. 3: Azimuthal integrated patterns of samples analyzed at the µ-SPOT Beamline of the BESSY synchrotron facility. In inset, the diffraction patterns of the abiotic magnetite control are presented.

Indeed, while the synthetic magnetite shows a reduction of the lattice parameter after the treatment, the treated magnetosomes exhibit an increased lattice parameter when compared to the original magnetosomes. Thus, besides side and surface effects that cannot be neglected so far, it seems that the magnetosome membrane not only serves as biological factory for the proteins responsible of magnetite formation, but also might play an unexpected mechanical role over the encapsulated biogenic magnetite nanocrystals.

Biomimetic Materials

Several putative magnetite biomineralizing proteins are found within the magnetosome membrane and/or attached to the crystals. Their respective roles are unclear as most show no or little homologies with other proteins from non-magnetic organisms. The protein MamJ is known to mediate the assembly of magnetosomes in vivo **[4]**. However, MamJ is an acidic protein that might interact with iron ions in vitro thereby affecting the synthesis of magnetite. Thus, recombinant MamJ proteins are currently investigated in vitro regarding their potential effects on magnetite crystal growth, size and morphology. Moreover, we are interested in the arrangement of the magnetic particles. 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, overexpression and isolation of MamK are currently underway to aid physical patterning of the biomimetic nanoparticles.

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From Microstructure to Mechanical Function

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This section reports results from external collaborations on biological and biomimetic materials, as well the work of several independent postdoctoral researchers: John Dunlop, Humboldt Fellow working on the development of internal stresses in biological tissues, Paul Zaslansky studying the three-dimensional microstructure of teeth and Notburga Gierlinger, an APART fellow studying plant cell walls as inspiration for nanocomposites.

Biomimetic materials research is a rapidly growing field **[1]** where design principles of natural materials are studied, modelled and used to imagine new types of artificial materials. A wide range of topics is covered, for example, in a special issue of Advanced Materials co-edited with Joanna Aizenberg from Harvard University **[2]**.

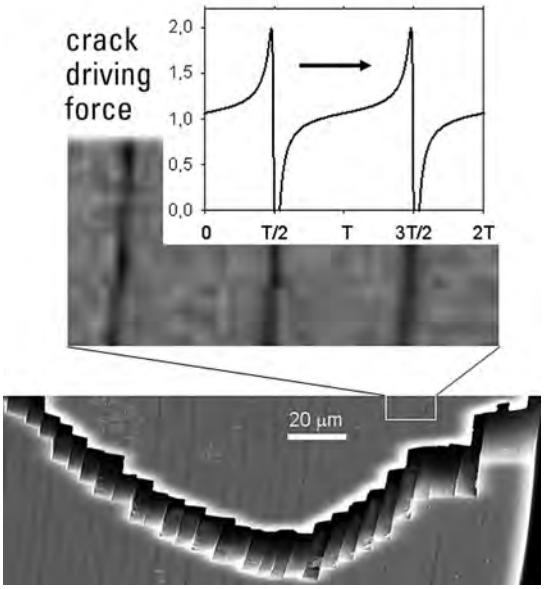

Fig. 1: The driving force for a crack propagating in a multilayered material with periodically varying elastic modulus is vanishing close to the minimum of the modulus. The example above shows a layered bioglass spicule with a series thin protein interlayers (dark grey). The calculation is done for a modulus ratio of 6 between the stiff and the soft layers [4]. The arrow shows the propagation direction of the crack.

In the years 2007/08, the hierarchical structure and the mechanical properties of silica sponges were continued to be studied in an ongoing collaborative project with Joanna Aizenberg and with colleagues at UC Santa Barbara **[3, 4]**. Additional details of the sponge skeleton architecture were discovered **[3]** and the fracture behaviour was analysed using an indentation method **[4]**. Cracks were seen to be stopped at the protein interfaces separating concentric silica layers in the spicule (see also bottom of **Fig. 1**). In this way, the inherent brittleness of glass is dramatically reduced, an effect which might be quite interesting from a practical point of view. This toughening principle was analysed theoretically together with the group of Dieter Fischer from the University of Leoben (Austria), currently Humboldt Senior Fellow in the Department. Using fracture mechanics concepts, it was shown that the crack driving force in a material with periodically varying elastic modulus may vanish close to the minimum of the modulus **[5]**. This means that a crack would effectively stop in the soft layer before a new crack is nucleated in the next layer, which also explains the stepwise propagation of the crack in silica spicules **(Fig. 1)**.

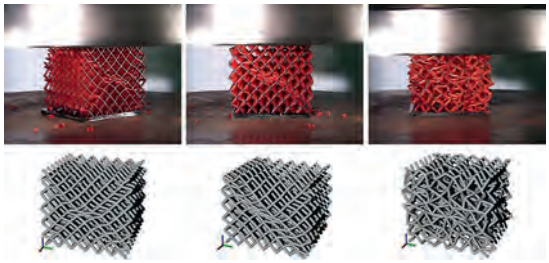

Fig. 2: Strut architectures built by rapid prototyping (top) or in the computer (bottom). Mechanical compression leads to strain localisation that depends on the degree of disorder in the strut arrangement [6]

Numerical modelling was also used in another project carried out in collaboration with the Vienna Technical University. Materials based on strut architectures with different degrees of randomness were built with rapid prototyping and their mechanical behaviour tested experimentally **(Fig. 2 top)**. In addition, deformation behaviour was simulated by a numerical model **(Fig. 2 bottom)**. It was shown that the major reason for strut failure is strain localization in shear bands (very well visible in **Fig. 2**) and that the localization is reduced with increasing randomness in the structure **[6]**. Such considerations are of great importance for the understanding of osteoporotic fractures in human vertebra, for example.

Finally, the structural basis and the mechanism of the movement of wheat awns **[7]** have been further studied in collaboration with Rivka Elbaum, a former Humboldt Fellow in the Department and now at Hebrew University in Israel. These awns perform a sort of swimming movement with cyclically changing air humidity by motor cells which expand or contract passively as a result of air humidity. It was shown that these motor cells have a multilayered cell wall structure **(Fig. 3)** with alternating cellulose fibril orientations in each layer **[8]**. The microscopic mechanism of the actuation turns out to be strongly related to these fibril angles with respect to the cell axis **[9]**. Depending on the fibril angle distribution in the cell wall, individual cells are expected to either shrink or expand in longitudinal direction. The (compressive or tensile) force also depends largely on this angle **[9]**

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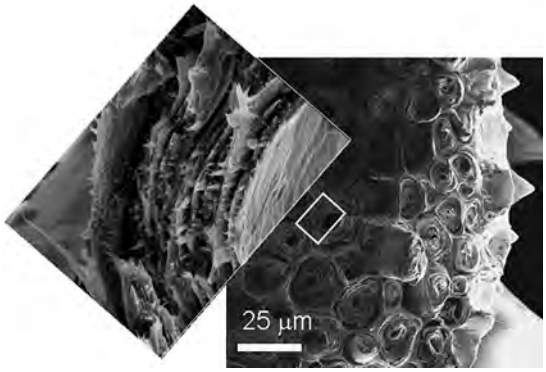

Fig. 3: Scanning electron microscopic picture of the actuating part of a wheat awn. The enlargement (left) shows the multilayered structure of the cell wall.

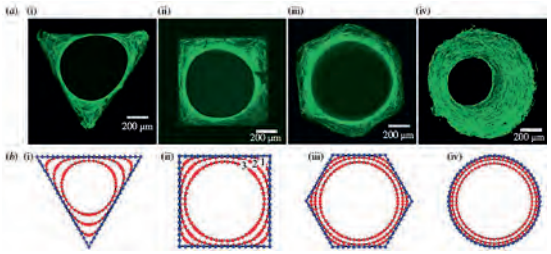

Fig. 4: (a) Tissue formed in three-dimensional channels (with actin fibres stained) after 21 days (i–iii) and (iv) 30 days of cell culture. (b) Numerical simulation of tissue formation within channels of various shapes. The lines (early time point 1, ongoing times 2 and 3) mark the simulated development of tissue formation (from [10]).

Modeling Stresses in Tissue and Tissue Growth

The work on the geometric control of tissue growth and stress development in plant organs discussed in the following is to be continued and expanded in the new research group Biomimetic Actuation and Tissue Growth, started at the end of 2008.

Recent studies have shown that in addition to biochemical signals, cells can respond also to physical signals such as the stiffness and shape of their environment. Research done in the Bone Regeneration Group of Manjubala Inderchand has shown that the rate of cell proliferation and new tissue growth in osteoblast cultures depends on the geometry of the environment in which the cells are growing. Osteoblast cell cultures were run in scaffolds of different channel shapes and showed that regions of higher negative curvature promoted more tissue growth than those of smaller curvature. This suggested that the growth process is controlled by local curvature, a well known phenomenon in materials physics. Simple simulations of curvature controlled tissue growth were run and closely matched the tissue growth patterns seen in experiment **[10]**. In particular, even though the local growth rate was geometry dependent, the global growth rate was found to be independent of shape, in both experiment and numerical simulations. These results may seem to be somewhat contradictory to the idea of curvature driven growth, however as the average mean-curvature of the prismatic channels tested are all the same then the average growth rate is independent. This is of particular importance in the design of scaffold materials for bone regeneration in addition to improving the understanding of the process of bone remodelling and fracture healing. This work is currently being extended (in collaboration with Prof. Dieter Fischer) to account for the coupling of stresses which develop in the tissue during growth.

Materials that can actuate complex motion or develop high stresses are particularly interesting with respect to potential application in MEMS, valves, artificial muscles and microfluidic systems. Of technical interest are the passive actuation systems found in plants which are mainly based on dead tissue. One example that can generate high stresses due to shape changes are the tension wood fibres found in the upper parts of branches of hardwoods studied in the Plant Biomechanics Group of Ingo Burgert. In many species the lumens of the tension wood cells are almost completely filled with an extra layer of parallel oriented cellulose (the G-layer), with the outside cell wall consisting of spirally wound cellulose. We were interested in understanding the stress-strain curve of tension wood before and after enzymatic treatment to remove the G-layer **[11]**. Un-treated wood, displayed zigzag oscillations in stress, much akin to the Portevin Le Chatelier effect, which disappeared after removal of the G-layer. By considering the G-layer as a load bearing element only weakly bound by frictional constraints to the cell wall, we could model the oscillatory stress-strain response of the tissue. The weak binding of the G-layer to the cell wall supported the idea that the G-layer is responsible for tensile stress generation. Upon hydration the parallel G-layer fibres swell pushing against the outer cell wall. The circumferential stress is converted into a contraction of the cell along its length resulting in generation of a high tensile stress **[11]**.

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Studies of Human Teeth: 3D Structure-Function Relations

Much is still unknown about the interplay between structural variation within human teeth (dentine, enamel) and the long-term durability – with no remodelling/healing – in the oral cavity. This durability may depend to a large extent on the subtle variations in microstructure and elastic and fracture properties of dentine **[12]**. Recent advances in coherent X-ray imaging and tomography are allowing us to match microstructure findings obtained by 2D methods (light and electron microscopy, speckle interferometry) with 3D-bulk scattering analysis (small angle x-ray scattering).

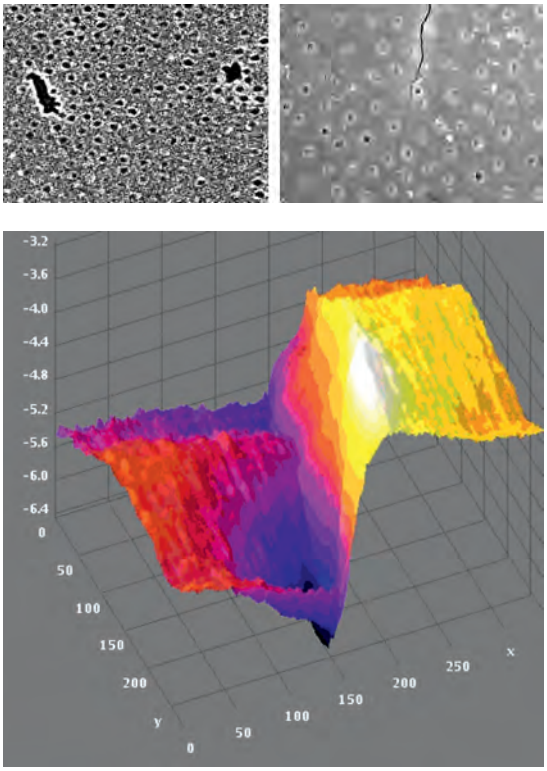

Fig 5: Phase-enhanced tomography slice in wet dentine (top left). Comparable information to that obtained by wet-mode SEM images of tubules with crack advancing under load (top right). Sequences of such images, combined with contrast-matching & statistical mage processing (wavelet-transform filtering) reveal nanometer displacement gradients increasing at and ahead of the crack tip seen in pseudo-3D displacement-magnitude profiles (bottom). Intertubular distance ~10 µm

Based on phase enhanced x-ray imaging (radiography) of dentine **[13]**, we are now able to resolve and find the spatial relationship between deforming zones in the tooth. As seen in **Fig. 5**, dentine tubules may be observed and tracked in slices in tomograms with submicron details (top left). This we hope to compare with environmental scanning electron microscope experiments of deforming and cracking wet dentine (top right). High resolution image-correlation analysis of images of the crack as it grows (bottom) reveal that the deformation process is non-linear, possibly due to the plasticizing effect of water.

To try and understand the extent to which water is involved in the deformation of teeth, neutron radiography and tomography contrast differences are being studied **(Fig. 6)**. Images of teeth immersed in D2O were compared with those of teeth immersed in deuterated methanol. Although limited by the moderate (supra-micron) resolution, preliminary results indicate that the deformation of the crown is constrained during the exchange of liquids. Tubules might be important for this. An asymmetric difference is seen in the right of **Fig. 6**, when tomograms of dehydrated teeth are subtracted from those of hydrated teeth, indicating that a difference exists in the contrast and scattering density of dehydrated roots. An asymmetric distribution of water around the root may be important for the mechanical functioning of the whole tooth.

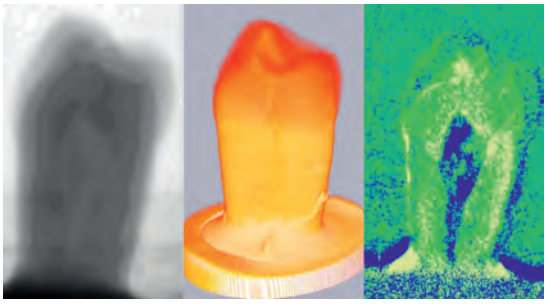

Fig. 6: Neutron radiography (left) and reconstructed tomography (centre) may be used to directly visualize the changes in contrast due to exchange of D2O and MethD4. Differential image (right) produced by numerical subtraction, shows bright yellow areas where water attenuation values were higher in the 'wet' state as compared with dark green and blue areas where attenuation is higher in the dry state. Much of the asymmetric difference is seen in the root section of the tooth.

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From Plant Cell Walls to Bio-inspired Composites

Plant cell walls are nanocomposites of cellulose microfibrils embedded in matrix polymers (pectins, hemicelluloses and lignin). As a result of adaptation to the different functional demands of plants, the cell wall polymers are arranged in many different patterns. The diversity in plant cell wall polymers and in their arrangement (cellulose microfibril orientation) results in biomaterials with very different properties. Thus investigating these "tuning parameters" is of importance to understand structure-function relationships and to learn from the broad range of nature´s plant cell walls. Confocal Raman microscopy (CRM) gives in situ insights into cell wall polymer composition and orientation with a high spatial resolution (<0.5 µm). The Raman imaging technique was during the last years successfully applied on different plant sources to reveal polymer compositions and orientations **[14-18] (Fig. 7)**.

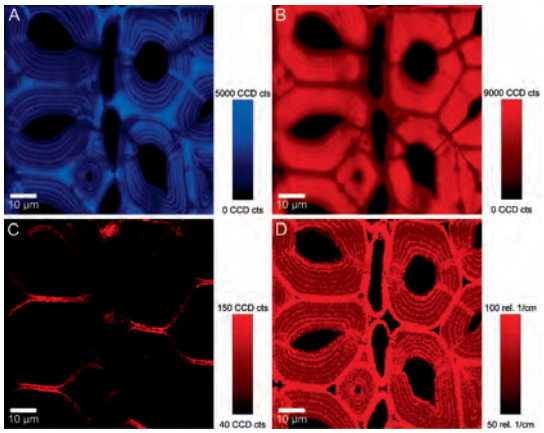

Fig. 7: Lignin distribution in the tropical tension wood (Laetia procera) shown by integrating from 1545-1698 cm-1 (A). Changes in cellulose amount and orientation visualised by integrating from 2774-3026 cm-1 (B), 1067-1106 cm-1 (C) and by plotting changes in band width from 2773-3044 (D)

Besides investigating the native cell wall, changes during tensile deformation **[19]** and enzymatic treatment **[20]** are of interest and can be followed by spectroscopic techniques. The development of a special designed fluidic cell by M. Schmitt, (Universität Heidelberg, in collaboration with Tillmann Rogge, Forschungszentrum Karlsruhe) allowed acquiring infrared spectra of biological samples in the wet stage and at controlled temperature as well as to exchange solutes. This enabled to follow for example in-situ the enzymatic degradation of the cellulosic G-layer in tension wood **[20]**.

Another approach is to build up cellulose nanocomposites by combining cellulose whiskers with different cell wall polymers and aiming to achieve preferred orientation for the cellulose whiskers **(Fig. 8)**.

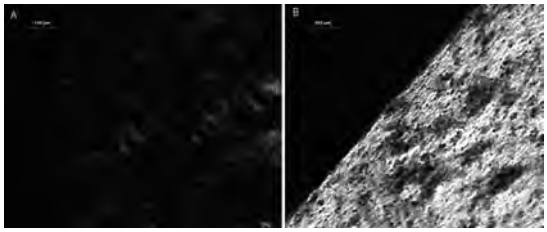

Fig. 8: Change in intensity of a light microscopic polarisation image (A) by rotating the sample 45°(B) as a hint for a preferred orientation in a cellulose/xyloglucan film.

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Biogenic Minerals and Bio-Inspired Nano-Composites

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Biological macromolecules play an important role in controlling the structure of biogenic minerals. Crystal size, shape and arrangement are modified by self-assembled organic matrices as well as soluble proteins. The resulting organic-inorganic composite structures have remarkable properties and hence constitute a rich source of inspiring concepts for the development of biomimetic materials. Besides hydroxyapatite, which is found in mammalian bone and teeth, calcium carbonate is a widespread biomineral that occurs in many marine invertebrates.

Biogenic and Biomimetic Calcium Carbonate

Biogenic calcium carbonate is of particular interest since it does not only cover a range of different morphologies, but also occurs in different polymorphs. Moreover, even the lattice parameters of biogenic calcite and aragonite were found to be anisotropically distorted, presumably due to the presence of intracrystalline proteins **[1]**.

Our research focused on studying the structure of intracrystalline organic inclusions with the aim of explaining differences between biogenic and geological calcite. In cooperation with E. Zolotoyabko (Technion, Haifa, IL) we investigated prismatic calcite crystals **(Fig. 1)** that were extracted from the shell of Pinna nobilis.

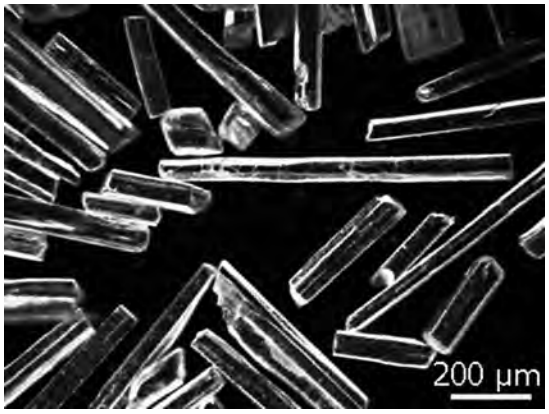

Fig. 1: Dark field light microscopy image of prismatic calcite crystals from a mollusk shell (Pinna nobilis).

Using a new experimental setup that was developed together with the group of O. Paris (Biomaterials Dept.) allowed for simultaneously studying the wide- and small-angle X-ray scattering behavior of single biogenic calcite crystals with a microfocus synchrotron beam at BESSY II (Berlin). **Fig. 2** shows an example for a 2-dimensional scattering pattern. The spots correspond to a single crystalline diffraction pattern of calcite. The small-angle scattering visible in the center, which arises from the organic inclusions, is anisotropic. As can be seen in the inset, which shows the small-angle region (000) in higher magnification, this anisotropy correlates with the crystallographic orientation.

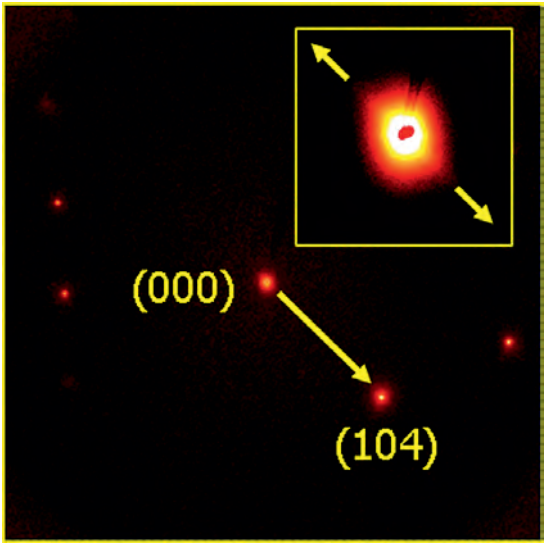

Fig. 2: Scattering of a single biogenic calcite crystal (Pinna nobilis). The anisotropic small-angle scattering (000) points towards the (104) orientation. The inset shows a higher magnification of the small-angle region.

A more detailed analysis showed that the organic inclusions are preferentially oriented not only along the {104} but also along the {001} crystallographic planes. Furthermore the small-angle scattering studies gave proof of the presence of a very rough internal interface between the organic inclusions and the surrounding mineral lattice. We assume that this large amount of interface is of major importance for controlling the properties of the biogenic mineral crystals.

Inspired by our findings on the structure of biogenic calcite, we performed similar investigations on biomimetic calcite that was precipitated in the presence of a soluble polymeric additive (in cooperation with H. Cölfen, Colloid Chemistry Dept.). Polystyrenesulfonate (PSS) was previously shown to induce the formation of calcite mesocrystals which consist of aligned nanocrystalline building blocks **[2]**.

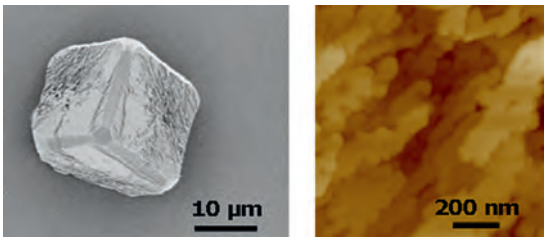

Fig. 3: SEM (left) and AFM image (right) of a calcite-PSS composite particle. The rounded corners of the particle belong to exposed {001} planes. The roughness of the surface can be seen in higher magnification in the AFM image.

The effect of the polymer on the morphology and the rough outer surface of the particles are shown in **Fig. 3**. The interaction with the polyelectrolyte favors the exposure of the charged {001} surfaces which appear additionally to the usually exposed low-energy {104} surfaces.

The characterization of these composite particles by means of X-ray scattering revealed several interesting structural features resembling the characteristics of biogenic calcite. The calcite-PSS particles appeared to be single-crystalline with polymer inclusions that are preferentially oriented, mainly along the {104} crystallographic planes **(Fig. 4)**. Moreover, the particles were also found to have a large amount of rough interface between the polymer and the mineral. This interface could be used to tune the properties of such composite particles.

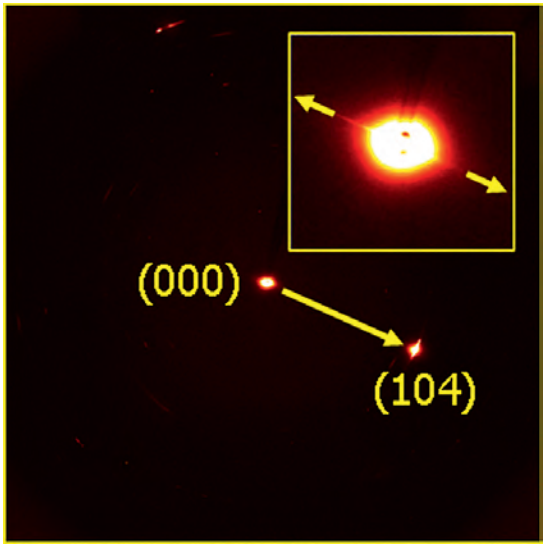

Fig. 4: Scattering of a single-crystalline calcite-PSS composite particle. The inset shows a higher magnification of the small-angle signal which points towards the (104) orientation.

The research was complemented by additional studies on Mg rich calcite from the tip of sea urchin teeth, together with Y. Ma and her coworkers from the Weizmann Institute of Science (Rehovot, IL). Specifically, the effect of Mg gradients and crystal orientations on the grinding capabilities and self-sharpening of the tooth were investigated **[3]**.

In the future, we will extend our research interests towards the development of biomimetic organic-inorganic composites with well controlled interfaces allowing for the combination of high stiffness with high toughness. Another aim will be to study the role of proteins for the formation of amorphous calcium carbonate in crayfish gastroliths (cooperation with A. Berman, BGU, Beer-Sheva, IL).

Mineralization of Tooth Enamel

Amelogenin proteins are the main component of the developing enamel tissue during its early stage of formation. In previous investigations **[4]** we analyzed the formation of so-called amelogenin "nanospheres" and showed an onset of their aggregation. This aggregation presumably leads to the formation of amelogenin chains that guide the growth of hydroxyapatite crystals during enamel mineralization.

Continuing these studies on the recombinant amelogenins rP172 and rM179 in cooperation with H. Margolis et al. (The Forsyth Institute, Boston, USA) **[5]**, we obtained more detailed information on the shape of the amelogenin nanoparticles which turned out to be oblates with an aspect ratio of 0.45. This was concluded from small-angle scattering measurements of protein suspensions **(Fig. 5)**.

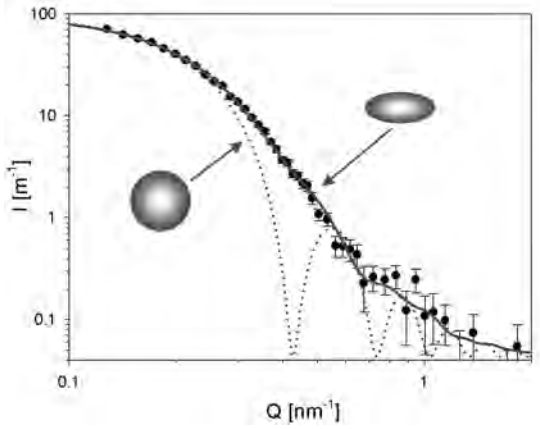

Fig. 5: Small-angle scattering profile (Intensity I vs. modulus of the scattering vector Q) of the recombinant amelogenin rP172 at pH8.1 and 4°C. The scattering is not consistent with monodisperse spheres (grey dotted line) but can be very well described by oblates (grey line).

The observed anisometric shape must be of crucial importance for the directed aggregation of the amelogenin particles into chain-like structures. Moreover, pH and temperature dependent measurements in different buffer solutions gave proof that the aggregation of (recombinant) amelogenin oblates occurs at a pH value of 7.2 which is close to physiological conditions.

Future studies will focus on the self-assembly behaviour of native amelogenins in order to evaluate the relevance of our results for the in-vivo formation of enamel.

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Mesoscale Materials and Synchrotron Research

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The research group has continued work on three different topics: 1) Further development and operation of the experimental station for simultaneous microbeam small- and wide-angle scattering (SAXS/WAXS) at the microfocus (µ-Spot) beamline at BESSY in BerlinAdlershof. 2) The comprehensive structural characterization of several biological materials which serve as inspiration source for biomimetic materials, and the attempt to transform some of them into potentially useful ceramics by combined thermochemical approaches. 3) The study of fluids in ordered mesoporous silica, with particular emphasis on the elastic deformation of the pore walls by the sorption and capillary condensation of the fluid. Much of the experimental work performed in 2) and 3) is based on position resolved and/or in-situ X-ray scattering at the BESSY instrument.

1) µ-Spot Beamline at BESSY

The SAXS/WAXS instrument at the BESSY µ-Spot beamline is fully operational since mid 2006 **[1]**. More than 40 experiments where conducted since then by user groups from the Biomaterials Department including some external cooperation partners. They were in all cases technically and in most cases also scientifically supported by our group. Some of the experiments will be described in more detail in the corresponding reports of other research groups or independent researchers from the department (Aichmayer, Burgert, Faivre, Gupta, Zaslansky), and only a short summary is given in the following. Microbeam scanning SAXS/WAXS is one of the most successful options of the BESSY instrument, allowing to construct maps of nanostructural parameters extracted from the SAXS/WAXS patterns with a resolution in the micrometer regime **[2]**. Examples of recent experiments on different types of biological materials include crustacean cuticles **(Fig. 1)**, plant cell walls, bone, and teeth.

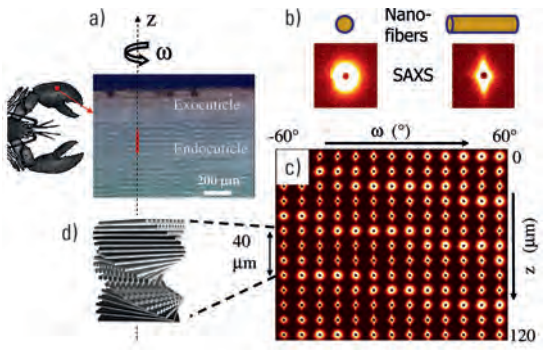

Fig. 1: 2D SAXS mapping of lobster cuticle nanostructure. a) Online light microscopy image of the specimen. b) SAXS patterns from chitin nanofibrils and their relation with fiber orientation. c) Composite image of SAXS patterns (10 µm beam) as a function of sample rotation angle ω and vertical sample position z. d) The characteristic change of the anisotropy of the SAXS patterns directly visualizes the rotated plywood structure of the chitin nanofibrils

Successful scanning SAXS/WAXS experiments were also conducted with external cooperation partners on sea urchin tooth (Weizmann Institute, Israel) and insect mandibles (Drexel University, Philadelpia). In close cooperation with the group of B. Aichmayer we have furthermore developed microbeam single crystal diffractometry combined with simultaneous 3D-SAXS. Here, the combination of microbeam SAXS/WAXS with full sample rotation is used for 3D reciprocal-space investigation of single crystalline or strongly textured particles of only a few microns size. Recent applications include single calcite particles of biogenic (cooperation: Technion, Haifa, Israel) and synthetic origin (cooperation Cölfen group, Colloid Chemistry) **[3]**, and on calcium phosphate particles (cooperation: Taubert group, Colloid Chemistry).

In-situ SAXS/WAXS are the second large group of experiments conducted at the BESSY instrument. Special devices have been developed and used for in-situ fluid sorption in mesoporous materials, for in-situ mechanical experiments on bone and other biological tissues, and for combined in-situ mechanical deformation and humidity control of plants. First approaches to combine in-situ experiments with microbeam scanning SAXS/WAXS have also been initiated by in-situ sample heating, combined with microbeam scanning of lobster cuticle (see below). Moreover, a first successful in-situ bending experiment combined with scanning from the tensile to the compression side of a lobster cuticle cross section was also conducted recently. Another in-situ experiment investigated mechanical creep of single carbon fibers of 10 µm diameter at high temperature (up to 2000°C) in cooperation with the University of Vienna.

2) Biological Materials and Biomimetic Processing

Crustaceans are known as the kings of mineral mobilization in the animal world. The crustacean cuticle is a nanocomposite consisting of chitin nanofibers associated with proteins and minerals, the latter being either calcite or amorphous calcium carbonate (ACC). The highly sophisticated hierarchical structure of the cuticle makes it an optimized material for mechanical protection and calcium storage. We have investigated the local nanostructure of lobster cuticle with scanning SAXS/WAXS, and have described the complex texture of the chitin fibers, as well as the crystallographic orientation relationship between chitin and the calcite mineral **[4]**. Moreover, we have shown that this calcite phase in lobster cuticle is restricted to a thin layer at the outermost exocuticle, while the rest of the cuticle contains exclusively ACC. We have attributed the function of the calcite layer to a mechanical protection role, in particular with respect to impact and wear resistance **[4]**. In a successive in-situ heating experiment we could show that the ACC phase transforms to calcite above 400°C, i.e. at a temperature exceeding the one of the biopolymer degradation by far **(Fig. 2)**. This allows speculating about the stabilization mechanisms of amorphous minerals, which is presently one of the key-questions in biomineralisation.

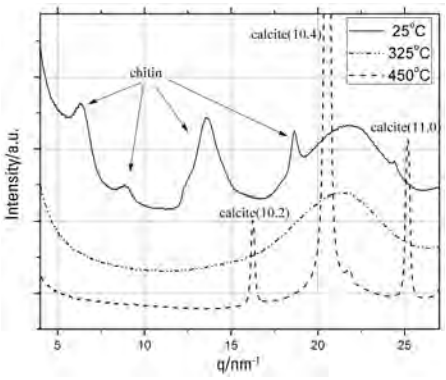

Fig. 2: WAXS profiles from lobster endocuticle as a function of temperature (taken from [4]). At 325°C, the crystalline chitin has fully decomposed and only a broad hump from ACC remains. At 450°C, almost all the amorphous mineral has been transformed into calcite.

Silica is one of the most abundant biominerals on earth besides calcium carbonate and calcium phosphate. In certain plants such as in rice husks for instance, considerable amounts of amorphous silica can be found in the outer epidermis. The functional role of silica in plants is however not yet clear. We have studied the structure of the perennial plant Equisetum hyemale (horsetail or scouring rush) with a series of complementary analytical techniques **[5]**. We could show that besides the known silica accumulations in particular knobs, the whole epidermis is covered by a thin silica layer. We attributed this to a mechanical protection role of silica for the plant body.

Besides the structural characterization, we have also attempted to isolate the biogenic silica from Equisetum hyemale **[6]**. Several chemical and thermal treatments were employed, and the structure and quality of the observed silica material was investigated by nitrogen sorption and small-angle X-ray scattering. Both, the long term treatment with hydrogen peroxide **(Fig. 3)** as well as short term treatment with hydrochloric acid followed by calcination revealed high quality mesoporous silica with large surface area (up to 400 m^2/g). Moreover, the macroscopic shape of the plant stalk could be perfectly preserved by the treatment **(Fig. 3)**. Therefore, this work opens new prospects for the production of high grade micro- and mesoporous silica from renewable resources.

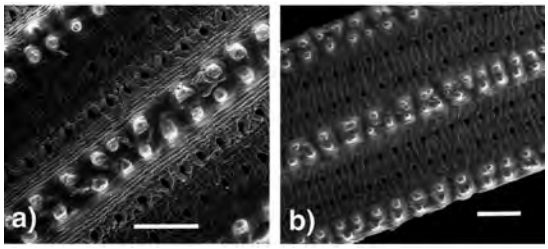

Fig. 3: Scanning electron micrographs of a native (a) and a long-term H_2O_2 treated sample of Equisetum hyemale (b). The sample in (b) consists of pure silica (taken from [6]). The length of the bars is 300 µm.

3) Fluids in Mesopores

In the framework of the Collaborative Research Center Sfb 448 "Mesoscopically Organized Composites", we have continued our work on in-situ sorption of fluids in ordered mesoporous silica using X-ray and neutron scattering. Moreover the development of simple physical models to describe the pore structure and the sorption process was also initiated. We have concentrated in particular on the deformation of the solid pore walls of the silica matrix during fluid sorption and condensation. These sorption strains can simply be obtained from the shift of the Bragg peaks from the ordered pore matrix in the used mesoporous materials SBA-15 and MCM41. The dependence of the strain on the fluid pressure at constant temperature ("sorption isotherm"), **Fig. 4** shows a continuous expansion during sorption, interrupted by a sudden contraction at capillary condensation. This behavior can be qualitatively understood by continuum thermodynamic and mechanical arguments **[7]**. Moreover numerical simulations performed by our cooperation partner from the TU Berlin show good agreement with the experimental data **[7]**.

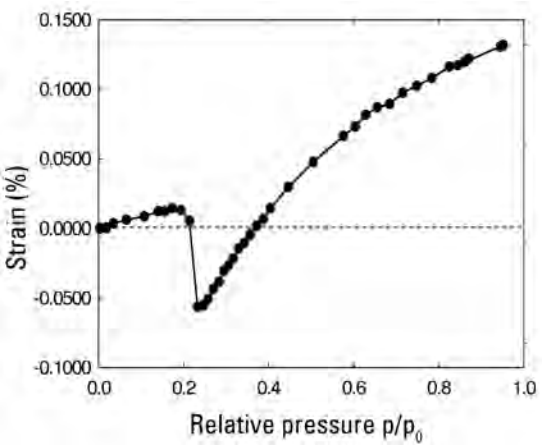

Fig. 4: Pore lattice strain of MCM-41 silica as a function of relative pressure of pentane at room temperature.

Strain isotherms as shown in **Fig. 4** were measured for different materials, for different pore diameters, and for different fluids. These data allow developing and refining sophisticated structural and mechanical models for these materials and lead to a better understanding of fluid-solid interactions in confined geometry. In addition, nanoelastic properties of the investigated materials can be estimated from these data in a unique way, which might be of great value for many novel mesoporous materials.

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