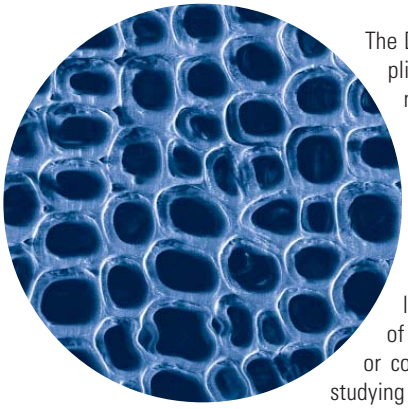
BIOMATERIALS

Research in the Department of Biomaterials



The Department of Biomaterials focuses on interdisciplinary research in the field of biological and biomimetic materials. The emphasis is on understanding how the mechanical or other physical properties are governed by structure and composition (see **Fig. 1**). Furthermore, research on natural materials (such as bone or wood) has potential applications in many fields. First, design concepts for new materials may be improved by learning from Nature. Second, the understanding of basic mechanisms by which the structure of bone or connective tissue is optimised opens the way for studying diseases and, thus, for contributing to diagnosis and development of treatment strategies. A third option is to use structures grown by Nature and transform them by physical or chemical treatment into technically relevant materials (biotemplating). Given the complexity of natural materials, new approaches for structural characterisation are needed. Some of these are further developed in the Department, in particular for studying hierarchical structures.

Hierarchical Structure of Natural Materials

The development of metals and alloys with increasing strength has been a constant trigger for the technical development of our societies. Interestingly, Nature does not use metals as structural materials at all. Practically all biological materials are based on polymers and polymer-mineral composites. The required mechanical performance is obtained by an intelligent structure which is hierarchical and optimised at all levels.

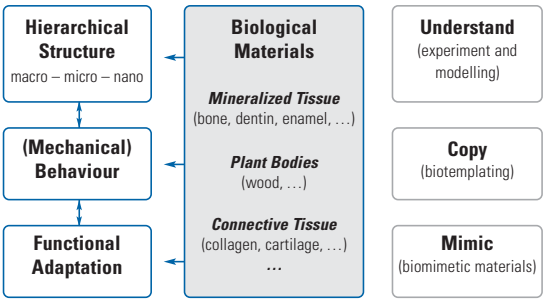


Fig. 1: General research goals in the Department of Biomaterials

Mechanical Adaptation of Biomaterials

It is also well-known that biological materials constantly adapt to changing mechanical needs. This is achieved by a strain-sensing mechanism, which in most biological systems is not fully elucidated. In the case of bone, for instance, specialized cells are thought to act as strain sensors and to be at the centre of a feed-back loop, called bone remodelling cycle, where damaged bone is removed and replaced by new material. This process is crucial for the tissue's capability of mechanical adaptation and self-repair.

New Methods for Analysis of Biomaterials

Studying hierarchical biomaterials requires state-of-the-art experimental equipment, but there is also some need for the development of new approaches. Scanning methods based on the diffraction of synchrotron radiation, as well as the technique of small-angle x-ray scattering (SAXS) are continuously developed to improve the characterization of hierarchical biomaterials. Further technical improvement is expected from a dedicated scanning set-up which is currently being installed at the synchrotron BESSY in Berlin.

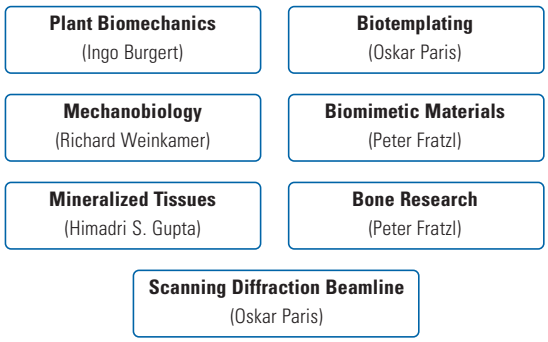


Fig. 2: Research groups in the Department of Biomaterials with respective group leaders

Research Strategy

The research on biomaterials is currently concentrated in seven research groups as sketched in **Fig. 2**. Three groups (left column in **Fig. 2**) deal with "understanding" (see **Fig. 1**) the mechanical properties of biological materials and their adaptation to external stimulus. Three more groups (right column in **Fig. 2**) concentrate on more applied goals, relating to the development of new materials, on the one hand, and to medical problems in bone research, on the other. Finally, a seventh group is dedicated to the development of a new micro-focus beamline for scanning x-ray scattering applications at the BESSY synchrotron in Berlin. More detailed goals of the different research groups are outlined below.

Plant Biomechanics

The main goals are:

- To understand plant tissue as a fibre composite in relation to its mechanical adaptation;
- To understand the nano-structure and mechanical properties of the plant cell wall;
- To obtain basic knowledge on structure-property relationships in plants for transfer to technical systems.

Mineralized Tissues

The main goals are:

- To understand how bone and related calcified tissues are designed at the micro- and nano- levels to fulfil their load-bearing and structural requirements;
- To develop a theoretical formulation that relates the mechanical properties of mineralized tissues to their structure at the sub-micron level.

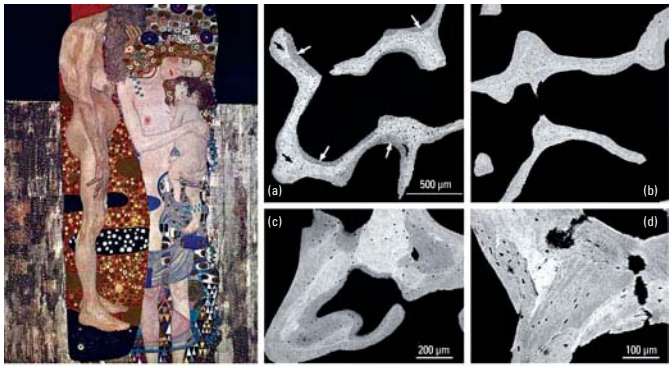
Mechanobiology
The main goals are:
- To understand and predict the adaptation of materials to mechanical requirements by means of numerical simulation;
- To improve the understanding of the biological response of natural tissues to mechanical stimulus and the associated feedback mechanisms by means of theoretical analysis.

Biotemplating
The main goals are:
- To take natural tissues as scaffolds or moulds for the creation of novel engineering materials with improved mechanical and functional properties;
- To preserve and/or replicate the hierarchical structure of the biological tissues down to the nanometer regime during the conversion process.

Biomimetic Materials
The main goals are:
- To use the building principles of natural hierarchical composites, such as bone, collagen or the plant cell wall, to improve existing materials by shaping and structuring.
- Current work includes: Biomimetic polymer-mineral composites; Designed porous scaffolds with optimised mechanical properties; Novel precious metal-based bio-nano-catalysts; synthesis of hydroxyapatite naoparticles with special shapes.

Bone and Mineral Research
This group works in close collaboration with external medical research groups and its main goals are:
- To study clinical problems related to bone material quality in various bone diseases, such as osteoporosis or brittle bone disease,
- To establish the effect of genotype on bone material in animal models and for genetic diseases,
- To develop new physical methodology for assessing bone material quality.

Scanning Diffraction Beamline
The main goals are:
- To develop a microfocus synchrotron beamline for scanning x-ray scattering applications, in collaboration with BESSY and the Federal Institute for Materials Research (BAM), the idea being to use the small-angle and/or diffraction signal to image a specimen with micrometer resolution;
- To develop high-throughput technology for online data analysis, to deal with the enormous amount of data collected in scanning diffraction applications;
- To implement a platform for the study of biological specimens (in particular cryo-sections).



Gustav Klimt (1862-1918):
Three ages of a women, 1905

(a) normal, (b) bisphosphonate, (c) parathyroid hormone, (d) NaF

Fig. 3: Various treatment strategies of osteoporosis investigated with back-scattered electron microscopy in collaboration with the Ludwig-Boltzmann Institute of Osteology in Vienna, Austria (courtesy, Dr. Paul Roschger)

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Calcified Tissue Structure and Mechanics

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Structural Adaptation and Mechanisms of Deformation in Calcified Tissues

The general aim of this group is to elucidate how the various structural features of natural calcified tissues (bone, tendon, cartilage) relate to their mechanical behavior **[1]**. Specifically, three types of tissues were addressed. (a) Mineralized cartilage, which is the crucial interface between subchondral bone and articular cartilage in articulating joints; (b) Parallel fibered mineralized collagen from tendon and bone, in order to elucidate mechanical deformation mechanisms at the fibril level; (c) And single osteons, the basic building block of compact bone, comprising layers of lamellar bone around a blood vessel. The aim was to resolve their intra-lamellar structure in terms of the orientation, size, shape and crystallographic structure of the nanometer size crystallites in relation to the mechanical properties of the lamellae.

Local Correlation of Modulus to Mineral Content at the Bone – Cartilage Interface

Patellar knee sections from human specimens were characterized mechanically and chemically, using scanning nanoindentation and quantitative backscattered electron imaging (qBEI), across the bone – calcified cartilage interface. Significantly different correlations between mineral content and elastoplastic properties were found between bone and calcified cartilage suggesting a different mineral particle – organic matrix arrangement at the fibrillar level. Quantitatively, our results are consistent with a model of thin elongated mineral particles tightly bound to an intermediate organic matrix **(Fig. 1a)**. At the tissue level, the generated two-dimensional material property maps of the elastoplastic properties **(Fig. 1b)** show that the bone cartilage interface is naturally designed as a functionally graded material, in order to minimize propagation of tissue – disrupting cracks, a finding which may have implications for biomedical engineers attempting to model the deformation and compressive behavior of articulating joints and their pathological alterations in common joint diseases like osteoarthritis **[1]**.

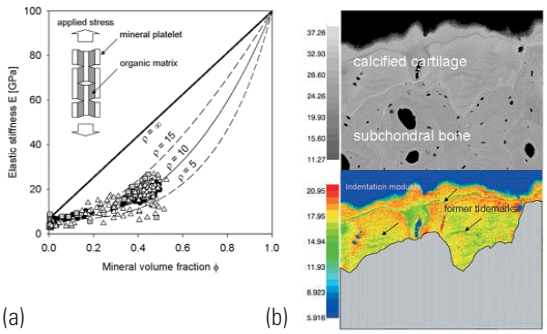

Fig. 1: (a) Comparison of the modulus–mineral relations predicted by the staggered model for ZCC with the measured cartilage nanoindentation modulus. Inset figure: schematic of the staggered model (b) Two dimensional property maps of calcium content and elastic modulus, at the bone – calcified cartilage interfaces

Fibrillar Level Deformation Mechanisms in Mineralized Tendons and Bone

For partially mineralized avian tendons for low (<1–2%) macroscopic strains, the fibril level deformation follows the applied external stress, for larger strains, an unexpected biphasic behavior is observed, where a portion of the fibrils relax back to their unstressed state while the remainder elongate to much larger strains, while maintaining macroscopic cohesion **(Fig. 2b)**. By combining the results with fractographic analysis using scanning electron microscopy, we find that the mineralized tendon consists, at the micrometer level, of a heterogeneously mineralized group of unmineralized and fully mineralized fiber bundles of 2 – 4 micron diameter. Our results are interpreted in terms of two-fiber composite model **(Fig. 2b)**, inset) in which the highly mineralized fibers account for the macroscopic stiffness and the lowly mineralized fibers the high work to fracture **[2]**. In contrast, our more recent work on parallel fibered bovine bone from the periosteum shows that the fibrillar strain in bone tissue is continuously proportional to that of the macroscopic strain. Surprisingly, this 1 – to – 1 correspondence is maintained in the inelastic regime, where mechanisms like microcracking of fibrils and fibril-matrix decohesion may be important **(Fig. 2c)**. The response of fibrils to stress and strain relaxation is the subject of current work.

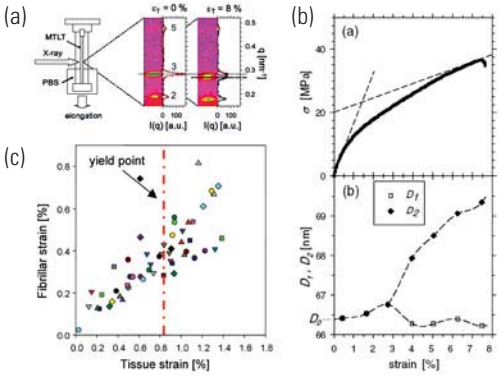

Fig. 2: (a) Principle of in-situ tensile testing combined with synchrotron X-ray diffraction. (b) Fibrillar level change in collagen D-periodicity to applied external strain in mineralized tendon. (c) Fibrillar level strain in bone compared to applied tissue strain

Mineral Particle Orientation and Nanomechanical Properties in Single Bone Lamellae

By combining µ-focus synchrotron X-ray diffraction and scattering (SAXS (small angle X-ray scattering) and WAXD (wide angle X-ray diffraction); **Fig. 3**) with texture measurements, we show quantitatively that the mineral platelets change their orientation, continuously, across a single lamella, consistent with the twisted rotated plywood model proposed by Wagner and Weiner. Indeed, our results permit, for the first time, reconstruction of the full 3D distribution of platelet and crystallographic orientations at a single point of 1 micron^3 volume. In combination with Raman microscopy measurements, our results will be used for a complete picture of the organic – inorganic structural and chemical composition within single bone lamellae.

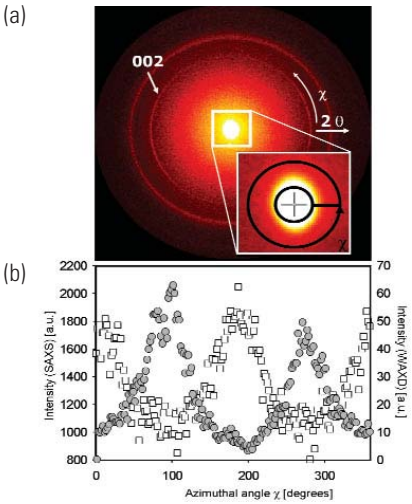

Fig. 3: (a) 2D MAR CCD detector image of osteonal bone, showing central SAXS signal and peripheral WAXD rings from (002) and (310) reflections (b) Integrated azimuthal SAXS and WAXD intensity profiles, with complementary information.

Scanning nanoindentation (500 µN, 20 µN/s) combined with backscattered electron imaging was used to build two – dimensional material property maps of the mechanical properties of human osteons **(Fig. 4a)**. It was found that each 5 micron wide lamellar unit consists of an alternately stiff and ductile layer, arising from a combination of the fiber orientation and mineral content **(Fig. 4b)**. This natural mechanism reproduces, at a lower length scale, similar results found for human dentin, and is likely to have a similar biological function **[3]**.

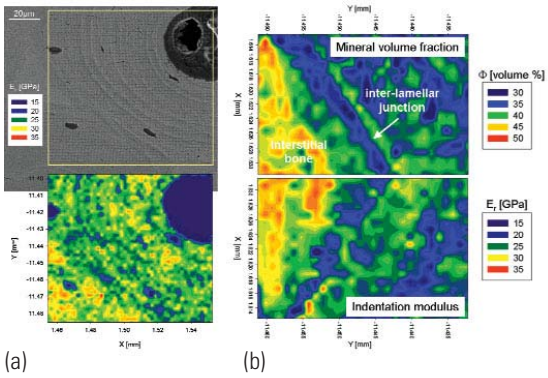

Fig. 4: (a) Two-dimensional map of elastic modulus around an osteon, showing the lamellar variation in mechanical properties (b) Detailed image of the edge of another osteon, where the correlation between mineral conten (top) and elastic modulus (bottom) is shown.

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References:

[1] Fratzl, P., Gupta, H.S., Paschalis, E.P., Roschger, P.: Structure and mechanical quality of the collagen-mineral nano-composite in bone. J. Mater. Chem. **14**, 2115-2123 (2004).

[2] Zizak, I., Roschger, P., Paris, O., Misof, B.M., Berzlanovich, A., Bernstorff, S., Amenitsch, H., Klaushofer, K., Fratzl, P.: Characteristics of mineral particles in the human bone-cartilage interface. J. Struct. Biol. **141**, 208-217 (2003).

[3] Gupta, H.S., Schratter, S., Tesch, W., Roschger, P., Berzlanovich, A., Schoeberl, T., Klaushofer, K. and Fratzl, P.: Two different correlations between nanoindentation modulus and mineral content in the bone-cartilage interface. J. Struct. Biol. **149**, 138-148 (2005).

[4] Gupta, H.S., Misof, K., Zizak, I., Meßmer, P., Roschger, P., Bernstorff, S., Klaushofer, K., Fratzl, P.: Mineralized microstructure of calcified avian tendons: a scanning small-angle x-ray scattering study. Calcif. Tissue Int. **72**, 567-576 (2003).

[5] Gupta, H.S., Messmer, P., Roschger, P., Bernstorff, S., Klaushofer, K. and Fratzl, P.: Synchrotron diffraction study of deformation mechanisms in mineralized tendon. Phys. Rev. Lett. **93**, 158101-1 (2004).

[6] Stachewicz, U.: Mechanical mapping of compact bone with lamellar resolution. Diplomarbeit, Max Planck Institute of Colloids and Interfaces (2004).

Bone and Mineral Research

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1988 and 1989: Visiting Professor (Rutgers University, New Jersey, USA)
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Thesis: Precipitation in alloys – small-angle x-ray scattering and computer simulation
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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)
Since 2004: Honorary Professor of Physics at Humboldt University Berlin

Bone is a hierarchical material and its mechanical properties depend on its structure at all levels of hierarchy. As a consequence, bone fragility may result from defects in any of the hierarchical levels. This concerns bone diameter and bone mass, as well as its internal architecture. At the lowest structural level, bone fragility could result from modifications in the collagen-mineral composite which constitutes the bulk of bone material. While bone mass is routinely evaluated in clinical practice, the quality of the bone material is much more difficult to assess. The general aim of this research group, which is constituted by researchers from the MPI as well as from the Ludwig Boltzmann Institute of Osteology in Vienna, a medical bone research institution, is to study changes in bone material quality with disease and treatment.

The main difficulty in this task is the extreme heterogeneity of the tissue which due to the permanent remodelling of bone. Old bone is being removed and new osteoid is added. This osteoid then mineralizes and the mineral content increases over several months. As a consequence, newly formed bone is less mineralized than old bone, as visualized, e.g., by back-scattered electron imaging in **Fig. 1**. In addition, mineral particles increase in thickness T from newly formed to mature bone **(Fig. 1)**.

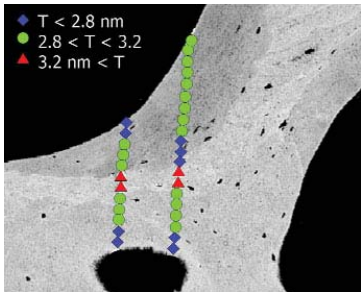

Fig. 1: Backscattered electron image of a section of human trabecular bone (from an iliac crest biopsy). The grey scale indicates mineral content, older bone being lighter than young bone. The black regions correspond to bone marrow and to cells embedded in bone. Young bone also has smaller mineral particle thickness T, as measured by small-angle scattering on the same section.

The consequence of this heterogeneity is that bone sections (usually from biopsies) have to be investigated in a position resolved way. The research group is developing and validating a number of techniques which all allow a resolution in the micron range and can be combined to study the same specimen: light microscopy to characterize cells and soft tissue components, backscattered electron imaging to determine mineral density distributions **[1]**, scanning small- and wide angle diffraction with synchrotron radiation to characterize mineral particles (see report about the beamline at BESSY) and scanning nanoindentation to study local variations of mechanical properties. The latest addition is Raman spectroscopic imaging, mainly to get information on the status of the organic component in fully mineralized bone.

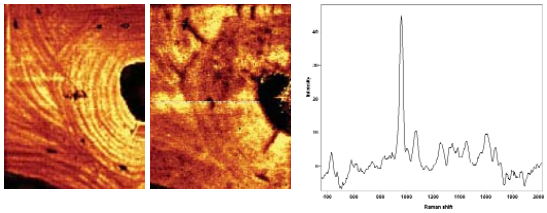

Fig. 2: (a) Fluorescence image and (b) Phosphate contrast of an osteon and (c) an example of a Raman spectrum from the pointed region.

Raman spectroscopy uniquely provides non-destructive, quantitative information simultaneously on the mineral and the protein matrix and is sensitive to local environmental effects, such as change in mineral substituents or protein secondary structures. Raman microspectroscopy and imaging provide molecular structure information with a spatial resolution in the micrometer range. The goal is to extract chemical information and spatial distribution without any prior information about the composition of the object being imaged. **Fig. 2** shows fluorescence and phosphate contrast Raman images of an osteon, that is, of the basic unit of compact bone consisting of a blood vessel surrounded by lamellar bone. The lamellar structure is clearly visible in the fluorescence image, but the phosphate distribution shows that mineral is homogeneously distributed across the lamellae.

Combinations of these approaches were used to study various cases of disease and treatment, both in animal models and in patient studies. Some examples are given below

Alkaline Phosphatase Deficiency

Tissue non-specific alkaline phosphatase (TNALP) is expressed in many tissues and is supposed to play an important role in bone mineralization. Reduced activity of this enzyme may lead to hypophosphatasia, a rare metabolic disorder. In order to get more insight into the importance of TNALP on the development of bone material, a transgenic mouse model deficient in this enzyme was studied **[2]**. A first interesting observation was that the texture of the bone material (that is, the alignment of the collagen fibrils and mineral particles) has a systematic variation along the cortical bone of the femur (see **Fig. 3**).

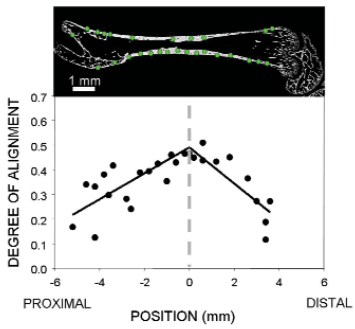

Fig. 3: degree of alignment of mineral particles in mouse femur (from [2]).

This orderly arrangement, which improves the bending strength of the femur, is lost in the TNALP deficient mice **(Fig. 4)**

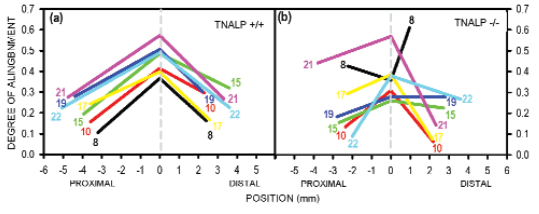

Fig. 4: Changes in the material texture with age and with TNAP status in mouse femur (from [2]).

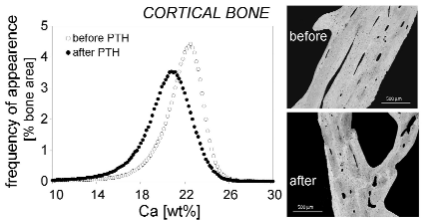

Fig. 6: The mineral density distribution is considerably broadened after PTH treatment [5].

Fra-1 Overexpression

Overexpression of Fra-1 results in an elevation of the number of mature osteoblasts, the bone forming cells. Bone material quality was studied in transgenic mice showing such an overexpression. While these mice are normal at birth, a dramatic increase in bone volume, well above normal levels (five fold!) occurs during maturation (see **Fig. 5**). Most interestingly, the material micro- and nano-structure did not show any obvious modifications which might encourage efforts to develop therapies based on Fra-1 against pathological bone loss **[3]**.

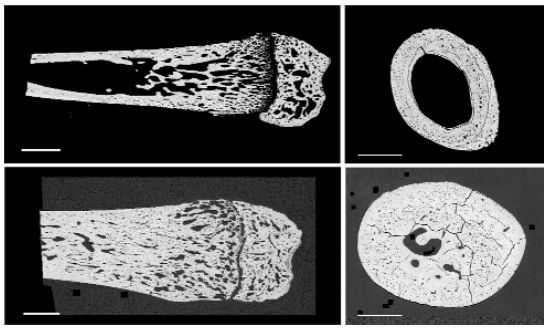

Fig. 5: Mineral distribution in femur or normal mice (top) and of transgenic mice with Fra-1 overexpression (bottom row) [3].

Vitamin D Receptor Overexpression

Vitamin D plays an important role in calcium homeostasis and in bone development. Bone material quality was investigated in transgenic mice with an overexpression of vitamin D receptors. The mineralization profile in these mice was more homogeneous than usual, however with normal structure at the nanometer level, a result which correlates well with the increased stiffness of bone in these animals **[4]**.

Osteoporosis Treatment with PTH

In a large international collaboration, the effect of parathyroid hormone (PTH) treatment on bone material was investigated for osteoporosis patients. Biopsies before and after treatment were investigated and showed a dramatic change in the mineralization pattern **[5]**, as visible in **Fig. 6**.

Pycnodysostosis

Pycnodysostosis is a rare genetic disease where patients are deficient in the enzyme cathepsin K, which is essential to degrade the bone matrix. As a consequence, bone remodelling is strongly disturbed. Biopsies from patients showed a disordered bone matrix, since a proper adaptation is not possible anymore. These results have importance beyond the actual disease, since cathepsin K inhibitors are currently investigated as possible drugs against osteoporosis **[6]**.

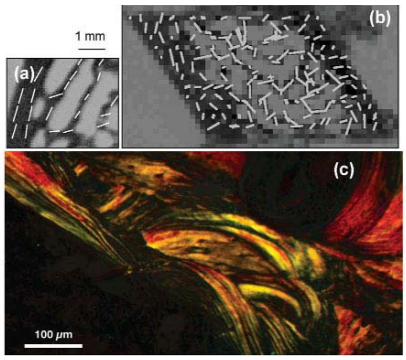

Fig. 7: Mineral particle orientation shown by white bars in (a) normal bone and (b) pyknodysostosis. (c) Disordered collagen arrangement revealed by polarized light (from [6]).

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References:

[1] Roschger, P., Gupta, H.S., Berzlanovich, A., Ittner, G., Dempster, D.W., Fratzl, P., Cosman, F., Parisien, M., Lindsay, R., Nieves, J.W., Klaushofer, K.: Constant mineralization density distribution in cancellous human bone. Bone **32**, 316-323 (2003).

[2] Tesch, W., VandenBos, T., Roschger, P., Fratzl-Zelman, N., Klaushofer, K., Beertsen, W., Fratzl, P.: Orientation of mineral crystallites and mineral density during skeletal development in mice deficient in tissue non-specific alkaline phosphatase. J. Bone Miner. Res. **18**, 117-125 (2003).

[3] Roschger, P., Matsuo, K., Misof, B.M., Tesch, W., Jochum, W., Wagner, E.F., Fratzl, P., Klaushofer, K.: Normal mineralization and nanostructure of sclerotic bone in mice overexpressing FRA-1. Bone **34**, 776-782 (2004).

[4] Misof, B.M., Roschger, P., Tesch, W., Baldock, P.A., Keckes, J., Meßmer, P., Eisman, J.A., Boskey, A.L., Gardiner, E.M., Fratzl, P., Klaushofer, K.: Targeted overexpression of vitamin D receptor in osteoblasts increases calcium concentration without affecting structural properties of bone mineral crystals. Calcif. Tissue Int. **73**, 251-257 (2003).

[5] Misof, B.M., Roschger, P., Cosman, F., Kurland, E.S., Tesch, W., Meßmer, P., Dempster, D.W., Nieves, J., Shane, E. , Fratzl, P., Klaushofer, K., Bilezikian, J., Lindsay R.: Effects of intermittent parathyroid hormone administration on bone mineralization density in iliac crest biopsies from patients with osteoporosis: a paired study before and after treatment. J. Clin. Endocrin. Metab. **88**, 1150-1159 (2003).

[6] Fratzl-Zelman, N., Valenta, A., Roschger, P., Nader, A., Gelb, B.D., Fratzl P., Klaushofer, K.: Decreased bone turnover and deterioration of bone structure in two cases of pycnodysostosis. J. Clin. Endocrin. Metab. **89**, 1538-1547 (2004).

Mechanobiology and Pattern Formation

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The premise of mechanobiology is that biological systems can detect mechanical stimuli and subsequently react to them. Examples for mechanobiological systems are plants and bone. We focused our attention to the remodeling process in trabecular bone (see **Fig. 1**) to understand how this process is controlled via the action of specialized cells.

In interplay of bone resorbing osteoclasts and bone depositing osteoblasts, living bone remodels its architecture in response to mechanical loading. This ability for adaptation is thought to occur in a remodeling process, where bone material is removed where the local mechanical stimulus is low and added where it is high (Wolff-Roux law). Implementing this law in a computer model and using finite-element methods (FEM), it was successfully demonstrated that optimized trabecular architecture emerges, is maintained and adapts to varying loads. However, concerning this mechanical feedback-loop the most basic questions are still unanswered like: to what mechanical stimulus cells are reacting to and in which way do they react? What is the connection between dysfunctions of the control system and bone diseases like osteoporosis? With computer simulations we can study the effect of different realizations of the control systems on the time evolution of bone microstructure **[1]**. An approximate, but fast algorithm to assess the local mechanical load in the network-like structure of trabecular bone was employed. The obtained result was then fed back into the local probabilities for bone deposition/resorption. This unknown law – we termed it remodel law – which couples the mechanical stimulus to the cell action is the basic unknown. The approximate treatment of the mechanics allows us to study the architectural evolution of trabecular bone inside a vertebra in a multitude of different settings and remodel laws over a human life time and beyond.

In our model the architecture of the trabecular bone inside a human vertebra is mapped on a simple cubic lattice with occupied sites, corresponding to bone, and unoccupied sites, corresponding to marrow. Such lattice models are successfully used for different problems in physics when geometry plays an important role **[2]** and the microstructures e.g., in alloys are often amazingly similar to the ones found in bone **[3]**. We assume that the local volume change is the mechanical stimulus the cells are responding to. As remodel laws simple relations between stimulus and deposition probability have been implemented, e.g., linear relations, step-functions or combinations of them as proposed in the bone literature.

Starting with a homogeneous configuration of high bone volume fraction, a network-like structure emerged with horizontal and vertical trabeculae **(Fig. 1)**.

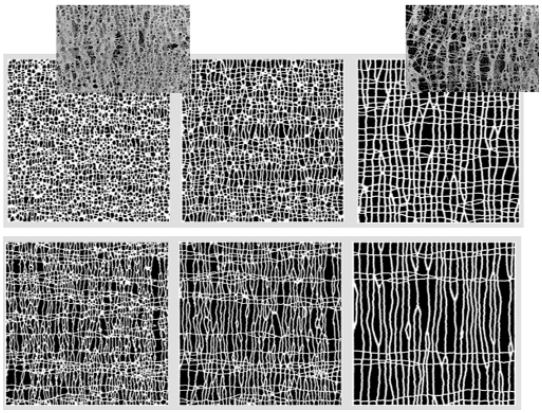

Fig. 1: Snapshots of two-dimensional simulations with time proceeding from left to right. Bone matrix is indicated white, marrow black. Simulations in the bottom row were performed with a reduced sensitivity of bone-depositing cells to the mechanical stimulus. For both simulations the starting configuration was a random arrangement of occupied sites of high volume fraction. The smaller, upper insets show for comparison micrographs of trabecular bone inside a human vertebra: young and healthy on the left, old and osteoporotic on the right.

In all simulations the bone volume fraction reached a steady state value. The architecture, however, coarsened, i.e., the trabecular number decreased while the trabecular thickness increased **[1]**. Since vertical trabeculae thickened faster, an anisotropy favoring the main loading direction became more pronounced. A small reduction in the sensitivity of the osteoblasts resulted in a decreased bone volume fraction and a deterioration of the microstructure from a mechanical point of view (**Fig. 1**, bottom row). These features of a reduced bone volume, a coarser structure and a more distinct anisotropy between vertical and horizontal trabeculae are observed also in osteoporotic patients.

While **Fig. 1** shows the effect of changes in the model parameter while the remodel law is fixed, **Fig. 2** compares the outcome of three-dimensional simulations using different remodel laws **[4]**.

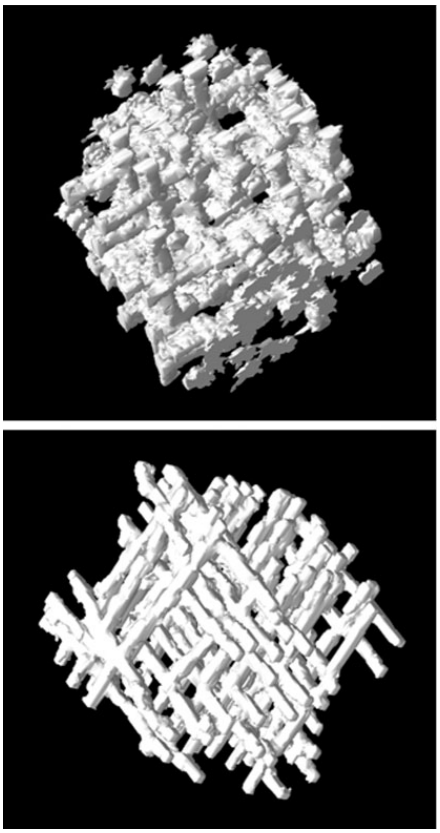

Fig. 2: Comparison between the three-dimensional configuration obtained by two different remodel laws: a linear relation between stimulus and deposition probability (top), a step-function corresponding to on/off-control (bottom).

The morphological differences can be quantified using either standard bone morphometry or more sensitive measures which we have developed. An influence of the remodel law can be observed not only in the morphology, but also in the effect of perturbations on the system. As an example we studied the effect of an increased turnover rate, i.e., a higher activity of the cells as observed, for instance, in women at menopause. In this case we obtained the result that depending on the remodel law the bone volume fraction either decreases or remains unaffected **[4]**. Interestingly, also in the case of an unchanged volume fraction the trabecular morphology coarses rapidly. All these observations have to be evaluated in comparison to data on real bone using advanced imaging techniques (e.g., µCT) and to outcomes from clinical studies. Based on this comparison we should be able to draw conclusions about the law governing bone remodeling.

As a final demonstration and future outlook of how this connection between simulation and experiments on bone works utilizing the "omniscience" in simulations, **Fig. 3** shows the age distribution in trabecular bone: younger bone is preferentially at the surface, older bone inside the trabeculae, as expected.

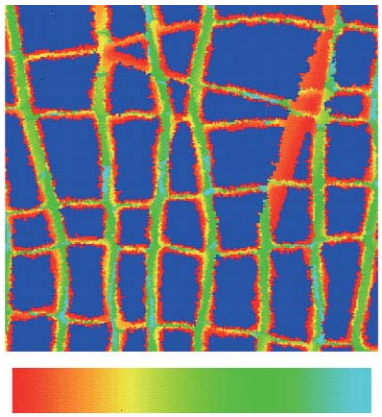

Fig. 3: A small cutout of the two-dimensional system showing the result of a representative simulation. The colors correspond to the time when the bone element was deposited. Red denotes the youngest (= most recently deposited) bone, turquoise the oldest bone. In real bone recently deposited bone can be detected as being less mineralized than older one.

Entering here the discussion about the possible creation of new trabeculae, the simulation demonstrates the existence of trabeculae consisting only of very young bone, but which were definitely not newly created. Since age corresponds to a higher mineralized state in real bone, this distribution is also experimentally accessible. A histogram of the bone mineral density was actually shown to be a sensitive fingerprint discriminating healthy and diseased bone.

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References:

[1] Weinkamer, R., Hartmann, M.A., Brechet, Y. and Fratzl, P.: Stochastic lattice model for bone remodeling and aging. Phys. Rev. Lett. **93**, 228102 (2004).

[2] Weinkamer, R., Fratzl, P., Gupta, H.S., Penrose, O. and Lebowitz, J.L.: Using kinetic Monte Carlo simulations to study phase separation in alloys. Phase Transitions **77**, 433-456 (2004).

[3] Weinkamer, R. and Fratzl, P.: By which mechanism does coarsening in phase-separating alloys proceed? Europhys. Lett. **61**, 261-267 (2003).

[4] Hartmann, M.A., Weinkamer, R., Brechet, Y. and Fratzl, P.: in preparation.

Plant Biomechanics – Structure-Function Relationships at the Micro- and Nanoscale

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Plants are hierarchically organised and possess remarkable mechanical properties. The unique performance of plant biomaterials is based on sustained optimization processes of the organism, which become obvious in the shape of the organs and in the adapted molecular structure. To meet the natural demands of a plant, the tissues are formed in various ways with respect to cell shape, thickness and arrangement of cell wall layers, orientation of the cellulose microfibrils as well as chemical composition. The basic assembly of plant cell wall structure are nanometer thick semi-crystalline cellulose fibrils embedded in amorphous matrix polymers. 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 the structure-function-relationships of plants at the micro- and nanoscale by carrying out microtensile tests combined with X-ray scattering, Raman spectroscopy, FT-IR microscopy and Environmental Scanning Electron microscopy.

Molecular Deformation Mechanisms

A) Slip-Stick Mechanism

Molecular deformation mechanisms of wet wood were studied by straining tissues and single fibres in a tensile stage monitoring stress response and collecting XRD signal using a two-dimensional (2D) CCD detector. Experiments were carried out at the ESRF Grenoble (European Synchrotron Radiation Facility). By relating stress-strain curves and XRD results, it was shown that the microfibril angle (MFA) of the cellulose fibrils significantly decreased with the applied strain.

In addition, further cyclic micro-tensile tests combined with video extensometry were performed in order to obtain stress-strain relations for individual cells and tissue foils in laboratory conditions.

At small deformations up to a yield point, a steep slope indicates a stiff material. Beyond the yield point, permanent deformation occurs without serious damage to the material since after releasing the stress, the original stiffness is recovered. The mechanical behaviour of individual cells is essentially the same as for intact tissue, except that intact tissue usually breaks at smaller strains. During cyclic loading, the stiff response at small stresses is always preserved in the region beyond the yield point **(Fig. 1)**.

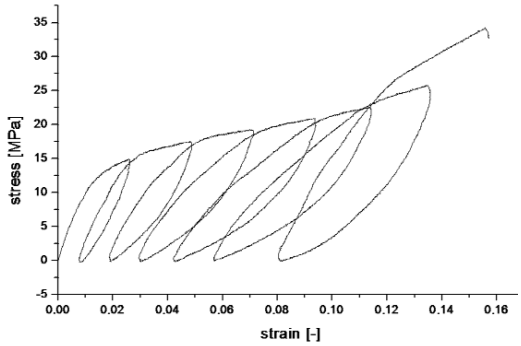

Fig. 1: Stress-strain diagram of wet compression wood tissue of spruce (MFA ~45°) in cyclic loading

Although, the structure of wood has nothing in common with that of metal the stress-strain behaviour of wood with high MFAs (e.g. compression wood) shows several characteristics that would normally be considered as typical for metals. The key properties of metals for their success as structural materials are their stiffness coupled to a reasonable plastic deformability, provided by the gliding of dislocations in the crystalline matrix.

The polymer assembly of the plant cell wall does not allow for a movement of dislocations. In our simple model for the deformation process, a large number of hydrogen bonds are able to transmit shear stresses between cellulose fibrils. When a certain shear stress is exceeded, the unspecific bonds break and there is a viscous flow of the matrix beyond the yield point. As soon as the stress is released, there is no back-flowing but a lock-in at the new position and the hydrogen bonds can reform immediately in the new position of the fibrils **(Fig. 2)**.

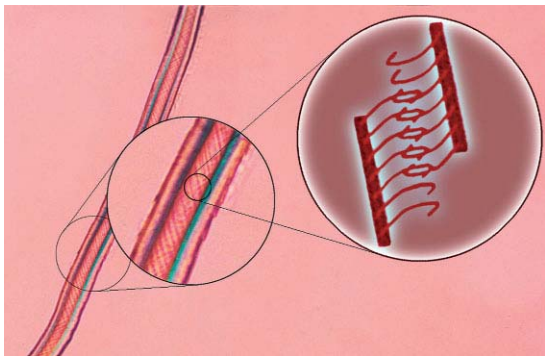

Fig. 2: Single compression wood tracheid in polarized light and schematic drawing of the "Velcro-connection" between the cellulose fibrils.

The plant biomaterial shows permanent plastic deformation without significant mechanical damage of the matrix, such as those usually observed in metals. The gliding of dislocations in metals, is replaced by a molecular stick-slip mechanism operated by some sort of "velcro" connection.

B) In-situ Raman Spectroscopy

A microtensile testing device was developed to strain thin plant tissue sheets and acquiring Raman spectra simultaneously. By relating stress-strain curves and changes in the Raman spectra, it is possible to evaluate molecular deformation mechanism as a function of external strain and strain rate. In a preliminary study 40 µm thick tangential sections of earlywood of pine were tested **(Fig. 3)**.

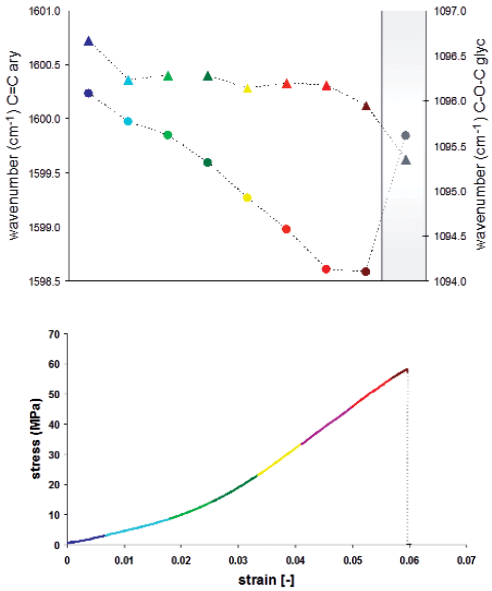

Fig. 3: Wavenumber shifts of C-O-C glyc (cellulose) and C=C aryl (lignin) at different stages of the tensile experiment. Curves and points are coloured according to the phases of tensile deformation in the stress-strain diagram (grey shadow = sample broken)

Spectra acquired during deformation show changes in peak intensity, peak shape and peak position. For instance, the band at 1095 cm^{-1} (C-O-C, glyc) corresponding to the stretching of cellulose is shifted progressively towards shorter wavenumbers **(Fig. 3)**, a demonstration that the cellulose molecule in these wood fibres are subjected to a uniform stress deformation. Almost no shift occurred for the 1600 cm^{-1} band corresponding to the amorphous lignin, which indicates although the lignin might be deformed, it is non-load bearing. Future in-situ tests will be applied to wood tissues with varying microfibril angle and polymer composition and will give new insights into deformation mechanism at the molecular level.

Specific Modification of Plant Cell Wall

A) Secondary Cell Wall Modification

The mechanical performance of plant cell walls 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 spatial orientation and bonding characteristics.

The basic idea of this project was to suppress the functioning of individual polymers in the mature cell wall of secondary xylem cells. Single fibres were isolated mechanically and the cell wall assembly was modified using specific enzymes. Micromechanical tests on the modified material were carried out to characterize its mechanical behavior without the missing component and thus, to learn more about the mechanical relevance of the eliminated polymer. Preliminary microtensile tests revealed the mechanical relevance of the eliminated polymers. Further enzyme treatments will target various hemicelluloses with the long term goal of developing a cell wall model based on the mechanical polymer interactions.

B) Primary Cell Wall Modification

A recent complementary approach to the foregoing project is to investigate structure-function relationships of primary plant cell wall components at the molecular level. In conjunction with the MPI for Molecular Plant Physiology (Lab. M. Pauly) we draw synergisms from the unique combination of plant physiology/enzymology/genetic engineering on one hand and micromechanical/ultrastructural characterization on the other. Harvested Arabidopsis hypocotyls are treated with various wall polysaccharide hydrolyzing enzymes to suppress the mechanical function of specific cell wall polymers. In a second approach hypocotyls are grown under the influence of the various enzymes. Micromechanical tests on the natural and on the modified hypocotyls from both approaches reveal the mechanical influence of the individual components, the interrelation of the polymer assembly, and potential compensation strategies of the plant.

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References:

[1] Keckes, J., Burgert, I., Frühmann, K., Müller, M., Kölln, K., Hamilton, M., Burghammer, M., Roth, S.V., Stanzl-Tschegg, S.E., Fratzl, P.: Cell-wall recovery after irreversible deformation of wood. Nature Materials **2**, 810-814 (2003).

[2] Burgert, I., Frühmann, K., Keckes, J., Fratzl, P., Stanzl-Tschegg, S.E.: Structure- function- relationships of four compression wood types - Micromechanical properties at the tissue and fibre level. Trees **18**, 480-485 (2004).

[3] Fratzl, P., Burgert, I., Gupta, H.S.: On the role of interface polymers for the mechanics of natural polymeric composites. Phys. Chem. Chem. Phys. **6**, 5575-5579 (2004).

[4] Burgert, I., Jungnikl, K.: Adaptive growth of gymnosperm branches-ultrastructural and micromechanical examinations. J. Plant Growth Regulation. **23**, 76-82 (2004).

[5] Fratzl, P., Burgert, I., Keckes, J.: Mechanical model for the deformation of the wood cell wall. Zeitschrift für Metallkunde **95**, 579-584 (2004).

Biotemplating

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Aims

Plant tissues can be used as scaffolds or moulds to design novel nanostructured inorganic materials. The challenge is to preserve or to replicate the entire hierarchical structure of the original tissues from macroscopic down to molecular length scales. Our actual research concentrates on the relationship between the local microstructure and mechanical properties of carbon materials from organic precursors, such as carbon fibres or monolithic carbon from wood and other plant tissues. An important question from a fundamental as well as from a practical viewpoint is, whether the cellulose microfibrillar orientation in the original plant cell walls can be transformed into a preferred orientation of the resulting carbon template. Other recent interests in our group relate to silica in plants with the prospects of direct synthesis of biomorphous SiC materials, and to nanocasting of wood by nanoparticle infiltration in order to synthesize novel functional ceramics.

Results

Carbon Fibers

Modern carbon fibres are based on polymeric- (polyacrylnitrile, PAN) or pitch-based precursors, and exhibit extremely high values of tensile strength and Young's modulus up to very high temperatures, making them superior low-weight materials for countless high-tech structural applications. However, the detailed microstructure and its development during the application of high loads as required for technical applications are still largely unknown. We have intensively investigated the local microstructure and its relation to mechanical properties in single carbon fibres by applying position resolved and in-situ diffraction techniques based on synchrotron radiation **[1,2]**. In a recent experiment **[3]** we combined in-situ bending of single carbon fibres with highest position resolution by scanning diffractometry across the bent fibres using a 100 nm sized X-ray beam provided by a waveguide structure. This experiment, performed at the microfocus beamline at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France, provided microstructural parameters such as the microstrain and the orientation of the carbon sheets depending on the local macroscopic strain in the compression and the tension zone of the bent fibre. As a major result, it was found that the neutral zone was shifted with respect to the centre of the fibre, which can be understood by a difference of the elastic modulus in compression and in tension. This difference can be explained by different orientation distributions of the carbon sheets under a specific loading pattern. In particular, strong buckling of the sheets in the compression regime could clearly be identified experimentally for some pitch-based fibres as compared to PAN-based fibres, indicating fundamental differences in the cross-linking of the sheets within the two fibre types. Further exploring the important role of these cross-links and to understand their physical origin is one of the future challenges of our work.

Wood Pyrolysis

Wood pyrolysis, i.e., the non-oxidative conversion of wood into charcoal has been extensively investigated from a chemical point of view, but not much is known about the structural development and the mechanical properties of the carbonaceous residue. Such knowledge is essential, however, if the material is used as a template for advanced composites based on the hierarchical structure of wood. We have therefore studied the structural development of the carbonaceous residue by combined small- and wide-angle X-ray scattering **[4]** and the local mechanical properties at the level of single cell walls by nanoindentation, both as a function of pyrolysis temperature T up to 2400°C. At least 5 regions with distinct differences in microstructural appearance and mechanical response can be distinguished (**Fig. 1**): i) degradation of the biopolymers and a decrease of the elastic modulus E to a very low value; ii) a fully disintegrated, amorphous structure at constant E; iii) formation and lateral growth of aromatic carbonaceous layer stacks as well as development of nanoporosity accompanied with a strong increase of E; iv) further lateral growth of carbon sheets at constant E; 3D growth of carbon "crystallites" and decreasing E. In particular, a preferred orientation of the carbon sheets parallel to the original wood cell axis develops with increasing T, indicating that the original cellulose molecular orientation might have been preserved.

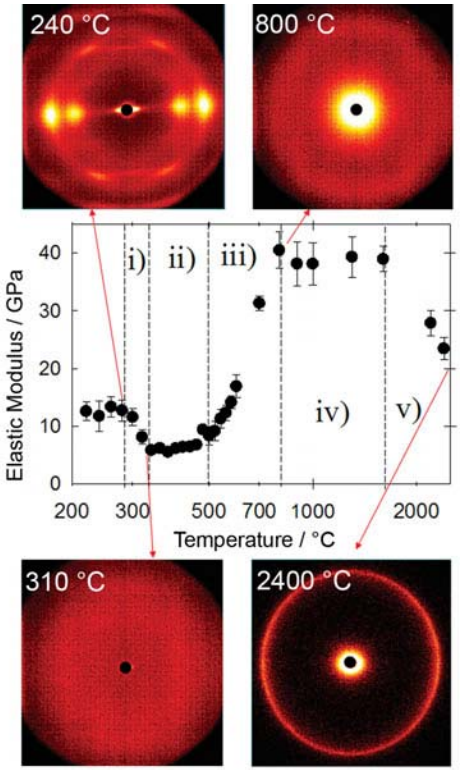

Fig. 1: 2D X-ray scattering patterns from pyrolysed spruce wood sections (cell axis vertical, range of d-spacings 0.25 nm - 6 nm), together with the temperature development of the reduced elastic modulus of the cell wall material.

The decrease of the modulus at high temperatures might be a consequence of this preferred orientation, since cell cross-sections were indented. However, the ultimate origin of the preferred carbon orientation, the mechanical response in terms of microstructure and chemical bonding, as well as the whole thermal conversion process are still to be explored in more detail.

Besides the carbonaceous residue as a biomorphous material, we are also interested in the details of the thermal degradation process of the biopolymers at the first stages of pyrolysis. To this end we have built a special heating device for in-situ X-ray scattering and have performed in-situ measurements of the kinetics of cellulose degradation up to temperatures of 400°C at the synchrotron radiation source HASYLAB (beamline A2) in Hamburg. The data of this recent experiment are currently still being evaluated.

Other Projects

Some annual plants contain considerable amounts of Si, mostly in the form of silica (up to 10 wt %), whose origin and biological function is still a matter of debate across several disciplines. Besides the potential use as a cheap and renewable source for direct SiC synthesis, the silica skeleton might be used as a scaffold for the synthesis of biomorphous ceramics. First studies on horsetail stalks (equisetum hyemale) show indeed that the ash of carefully calcined specimens replicates largely the original plant structure. Current work focuses on the detailed distribution of silica (see **Fig. 2**) as well as on its microstructural and chemical appearance.

Fig. 2: X-ray microCT reconstruction of a horsetail stalk (diameter of the stalk ≈ 3.5 mm). Red overlays are regions of particularly high X-ray absorption, indicating strong silica accumulations.

Another project that just started deals with the prospect of nanocasting plant cell walls by nanoparticle infiltration. The aim here is to copy the detailed cellulose microfibril orientation into a ceramic material. First synthesis approaches with CeO_2 nanoparticles have been undertaken, but no definitive positive templating results can be reported to date.

Two further projects which do not directly concentrate on biotemplating but rather on biomimetic/biomechanical aspects have recently been initiated. First attempts were undertaken to map the chitin orientation in the neighbourhood of mechanoreceptors in insect cuticles using scanning microbeam X-ray diffraction. The particular aim here is to understand the role of local fibre orientation on the function of integral strain sensors. Together with the "plant research group" in the Department of Biomaterials we furthermore started recently to explore the structure directing role and the dynamics of water in the nanopores of wood cell walls. In this context, a research proposal has been submitted within the Collaborative Research Center (SFB) 448 "Mesoscopically Organized Composites".

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References:

[1] Loidl, D., Peterlik, H., Müller, M., Riekel C. and Paris O.: Elastic moduli of nanocrystallites within carbon fibers measured by in-situ X-ray microbeam diffraction. Carbon **41**, 563-570 (2003).

[2] Loidl, D., Paris, O., Müller, M., Burghammer, M., Riekel, C., Kromp, K. and Peterlik, H.: Nanostructure of single carbon fibres investigated with synchrotron radiation. in: Nanotechnology in Construction, ed. by Bartos, P.J.M., Hughes, J.J., Trtik, P. and Zhu W., The Royal Society of Chemistry, Cambridge, UK (ISBN 0-85404623-2) pp 205-214 (2004).

[3] Loidl, D., Peterlik, H., Paris, O. and Riekel, C.: Local nanostructure of single carbon fibres during bending deformation. ESRF Highlights 2004, 49-50 (2005).

[4] Paris, O., Zollfrank, C. and Zickler, G.A.: Decomposition and carbonization of wood biopolymers – A microstructural study of softwood pyrolysis. Carbon **43**, 53-66 (2005).

Biomimetic Materials

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The general aim of this group is to use knowledge of the building principles of natural hierarchical composites, such as bone **[1,2]** and wood **[3]** to improve existing materials just by shaping and structuring. While chemical composition and supra-molecular structure are well-known to determine the material properties, biological systems demonstrate that mechanical and other properties can also be modulated in a wide range just by an appropriate geometrical arrangement of the material in space. Examples are spiralling fibre structures with varying spiral angle, as in the wood cell for example **[3]**, or the architecture of the cancellous bone in the interior of a vertebra **[2]**. One of the goals was to explore this paradigm, assuming that it should be possible to "tune" the mechanical properties of various porous materials (ceramics, polymers or composites) by controlling the pore architecture. A second goal was to develop porous scaffolds with controlled pore geometry for bone cell culturing. Most of this work was carried out in collaboration with the University of Technology and the Ludwig Boltzmann Institute of Osteology, both in Vienna, Austria.

Rapid Prototyping (RP)

In order to structure porous materials in the micron to millimetre range, rapid prototyping technology was established in the Department. The potentials of two different systems were explored, one based on photolithography and a second one based on inkjet printing. In both cases, three-dimensional structures are built layer by layer from a model constructed on a computer. The first process is shown schematically in **Fig. 1**: a layer of photosensitive resin is selectively exposed to visible light and polymerised. Three-dimensional structures are built by moving the building platform continuously upwards.

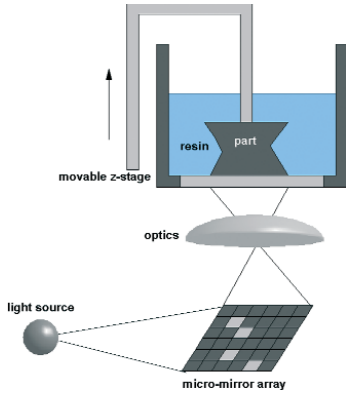

Fig. 1: The principle of rapid prototyping based on photolithography.

The second rapid prototyping process corresponds to a wax printer as sketched in **Fig. 2**. Three-dimensional structures are built by writing successive layers using two types of waxes, the (blue) building wax and the (red) support wax, which is later removed by a solvent. Both techniques allow free-form fabrication of arbitrary structures with a pixel resolution in the range of 30 microns.

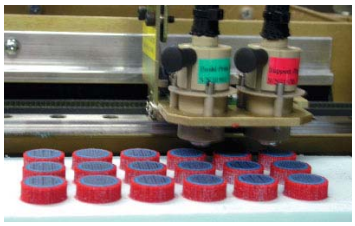

Fig. 2: Porous structures being built by inkjet printing using two types of waxes.

Mechanical Properties of Cellular Solids with Controlled Architecture

A number of porous structures with different internal architecture have been designed on the computer and built with rapid prototyping **(Fig. 3)**.

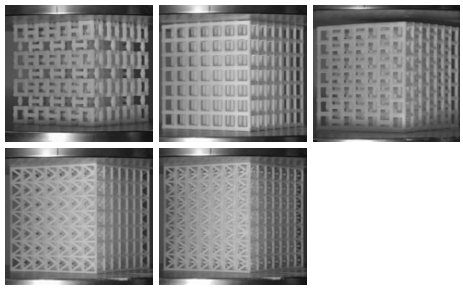

Fig. 3: Cellular Solids with constant apparent density, but varying architecture.

The idea was to test the influence of the internal geometry on the mechanical properties. Cubic specimens were designed with a given overall size, shape and apparent density, but with different internal architecture. The mechanical properties, such as stiffness or impact energy absorption efficiency, were observed to vary within at least a factor three **[4]**. This project is being continued by studying the orientation dependence of the properties, as well as the influence of special types of defects. Moreover, the performance of artificially designed structures will be compared to structures reconstructed from natural models such as cancellous bone using same resin as for the artificial structures.

Bioceramic Bone Replacement Materials

Using the RP equipment mentioned above, we produced casting moulds for cellular bone replacement materials. Using RP methods offers the possibility to produce almost arbitrary geometries, which can be beneficial not only from a mechanical point of view, but also from a biological point of view, as the cell ingrowth behaviour strongly depends on the geometry of the porosity of an implant. To produce ceramic structures with continuous pores with a diameter in the range of 500 microns, a polymer casting mould was first constructed by RP. The structure chosen is sketched in **Fig. 4** and corresponds to a "woodpile arrangement" with layers of parallel struts. The struts in two successive layers were oriented 90° to each other. In this structure, the hollow space has the same geometry as the filled space.

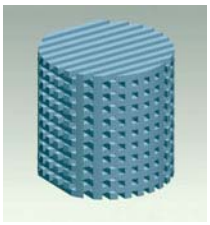

Fig. 4: woodpile structure as designed

This structure, covered with a mantle, was produced in resin as a casting mould for ceramic gel casting: Ceramic powder (we mostly used commercially available hydroxylapatite (HA) powder) was mixed with water, monomers and a polymerization initiator. Vacuum was used to fill the mould with the ceramic slurry. Polymerization occurred during a following thermal treatment, giving the cast part some strength. Further elevation of the temperature caused the water to evaporate, then the mould to burn off and, finally, led to sintering of the ceramic particles. Typical structures obtained by this process **[5]** can be seen in **Fig. 5**.

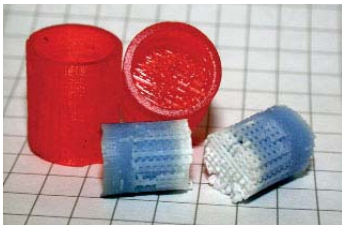

Fig. 5: resin casting moulds (red) and hydroxyapatite structures obtained by RP and ceramic gel casting

Bioceramic/Biopolymer Composite Bone Replacement Materials

In bone material, organic fibres (collagen) and mineral nanoparticles are combined at the nanometre scale. To mimic this situation, porous scaffolds were also made of a composite of the biopolymer chitosan with apatite particles. Chitosan has been used before as a matrix for three-dimensional tissue growth and is a potential candidate for tissue engineering and drug delivery systems. The composite scaffolds were produced by RP using dissolvable wax moulds. This was necessary since temperature treatment (to remove resin scaffolds produced by other RP techniques) was not possible due to the chitosan component in the scaffolds. The scaffolds were then freeze-dried and cross-linked to produce micro pores in addition to the macropores **(Fig. 6)**.

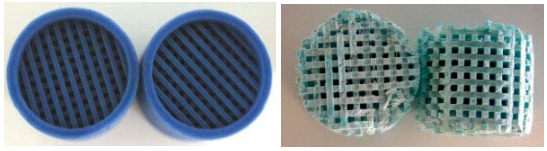

Fig. 6: wax moulds (left) and chitosan/HA composite scaffolds (right).

Biocompatibility of Bone Scaffolds:

The fabricated hydroxylapatite and chitosan/apatite scaffolds were assessed for their biocompatibility with bone cells using a pre-osteoblastic cell line, known to be able to differentiate into active osteoblasts. Cells were covering the scaffolds, sometimes in several cell layers, and they produced extra-cellular matrix in 3 weeks **[5]**, as seen from histological staining **(Fig. 7)**.

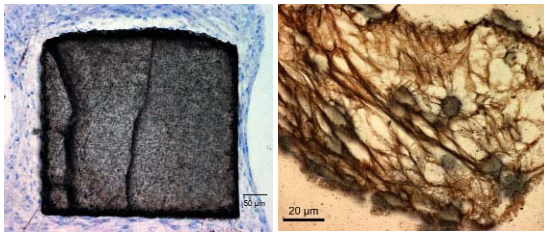

Fig. 7: Histological sections of the ceramic scaffolds after a culture period of 14 days. Giemsa staining shows cells covering the whole surface of a strut (left), Gömery staining reveals the formation of an extracellular matrix consisting of collagen (right).

Nanoparticles

Some activity was started to control the size and shape of hydroxylapatite (HA) nanoparticles by precipitation reactions involving use of specific ligands which can affect the nucleation and growth mechanism in addition to reaction parameters like precursors, solvent system, temperature and pH. The rationale is that the specific shape of the HA nanoparticles and their interaction with the organic component plays an important role in the mechanical properties of the biominerals. Additionally controlling the size and shape of the HA nanoparticles, their functionalisation and self-assembly to get materials with hierarchical structures is also interesting for various applications including bone implants, catalyst supports and radioactive waste management.

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References:

[1] Gao, H., Ji, B., Jäger, I., Arzt, E., Fratzl, P.: Materials become insensitive to flaws at nanoscale: lessons from nature. Proc. Natl. Acad. Sci. USA **100**, 5597-5600 (2003).

[2] Fratzl, P., Gupta, H. S., Paschalis, E.P., Roschger, P.: Structure and mechanical quality of the collagen-mineral nano-composite in bone. J. Mater. Chem. **14**, 2115-2123 (2004).

[3] Fratzl, P.: Cellulose and collagen: from fibres to tissues. Curr. Opin. Coll. Interf. Sci. **8**, 32-39 (2003).

[4] Woesz, A., Stampfl, J. und Fratzl, P.: Cellular Solids beyond the Apparent Density – an Experimental Assessment of Mechanical Properties. Adv. Eng. Mater. **6**, 134-138 (2004).

[5] Woesz, A., Rumpler, M., Stampfl, J., Varga, F., Fratzl-Zelman, N., Roschger, P., Klaushofer, K. und Fratzl, P.: Toward Bone Replacement Materials from Calcium Phosphates via Rapid Prototyping and Ceramic Gelcasting, Mater. Sci. Eng. C, in press (2005).

Synchrotron Beamline at BESSY

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Aims

Hierarchically structured (bio)materials are a central research topic in the Department. Such materials need to be characterized by a variety of methods and over many length scales. This cannot be achieved by conventional methods of structural analysis and requires further development of experimental techniques, for example, scanning small-angle (SAXS) and wide-angle X-ray scattering (WAXS), and corresponding data analysis methods **[1-5]**. Currently, we are developing a scientific instrument at the microfocus beamline at BESSY II in Berlin Adlershof, with the main goal to implement a scanning device for the combination of simultaneous SAXS, WAXS and X-ray fluorescence analysis (XRF). This unique combination of methods will allow to map structural parameters from the atomic/molecular to the nanometer level as well as chemical composition with a spatial resolution given by the beam size of a few micrometers. Since our long-term goal is to proceed from microbeam scanning to a real imaging technique, a major task is the development of sophisticated software tools for interactive instrument control combined with online data analysis, which is done in cooperation with partners from the European Synchrotron Radiation Facility (ESRF). For the preparation of biological specimens and the development of specimen platforms such as cryo-cooling techniques, a sample preparation laboratory is also under construction.

Beamline and Experimental Station Development

The microfocus beamline at BESSY is a joint project between the Max Planck Society, BESSY GmbH, and the Bundesanstalt für Materialforschung (BAM). The measuring station is developed in close collaboration with these partners and with the Technical University Berlin. The beamline was built by an external company (ACCEL) and has been installed in December 2004 as a second branch of the 7 T wavelength-shifter of the BAMLine at BESSY II. The final beamline commissioning is scheduled for April 2005. For the SAXS/WAXS/XRF experimental station, five fixed energies (4-24 keV) from a combined Bragg-Fresnel - bimorph mirror system with a beam divergence < 1 mrad, a beam size ≤ 5 µm and a photon flux $\geq 10^9$ ph/s will be available. The principal design of the station **(Fig. 1)** was developed during 2004, and the main components have been purchased.

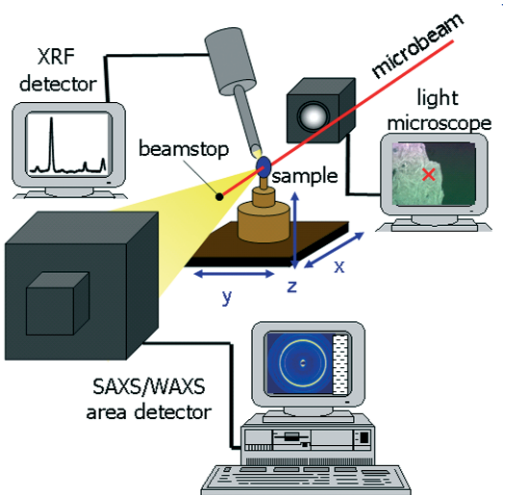

Fig. 1: Schematic layout of the SAXS/WAXS/XRF experimental station at BESSY

Cornerstones of the experimental setup are a flexible and modular goniometer with a high precision scanning stage (0.2 µm resolution), a high-resolution, on-axis optical microscope, and a high-resolution, large-area CCD detector with fibre-optic taper (MarMosaic). Using this detector with the given small size and divergence of the beam, up to three orders of magnitude in the length of the scattering vector q can be covered simultaneously, and thus, SAXS/WAXS with a single detector and a resolution down to $q = 0.1$ nm^{-1} will be feasible ($q=4\pi/\lambda\sin(\theta)$, where 2θ is the scattering angle and λ is the wavelength). The installation of the experimental station is scheduled for April 2005 and first test experiments can be anticipated for Summer 2005.

Online Data Analysis

Scanning SAXS/WAXS experiments produce 2D scattering patterns as a function of (at least) two scanning coordinates. This results typically in many thousands of 2D patterns, and consequently, data reduction and analysis needs to be at least partially automated. Moreover, it is highly desirable to get an overview of the progress of an experiment (e.g., to decide about follow-up measuring strategies), and therefore, the experimental setup should allow for a online mapping of some selected simple parameters deduced from the scattering patterns. In the long-term, online mapping of microstructural and chemical parameters derived from SAXS/WAXS/XRF data by pre-defined automated data reduction and -evaluation procedures should bring the technique eventually to a similar level as current scanning electron- or scanning probe microscopy techniques.

In the framework of a long-term project at the ESRF (2004-2006, LT-proposal SC-1579), three European laboratories from MPI-Golm, ESRF-Grenoble, and CITER-Cardiff have constituted a research consortium on "Scanning X-ray diffraction of hierarchical biological tissues". An important common goal of the consortium is to develop a software platform for interactive instrument control and online data-reduction and -analysis. The actual version of the package consists of a PYTHON-based "toolkit" developed by M. Burghammer (ESRF), interfacing with the instrument and with sophisticated data analysis programs such as FIT2D. In the future, this package will be continuously improved by the partners and will be implemented at the experimental station at BESSY.

A preliminary version of the software was successfully tested during the first beamtime of the LT-proposal in November 2005. Several biological materials such as osteonal bone, insect cuticle or eggshell were investigated, and "simple" parameters derived from the 2D patterns such as the total scattered intensity were mapped online. **Fig. 2** shows an example of osteonal bone, where the total SAXS intensity clearly reflects the lamellar structure of the osteon.

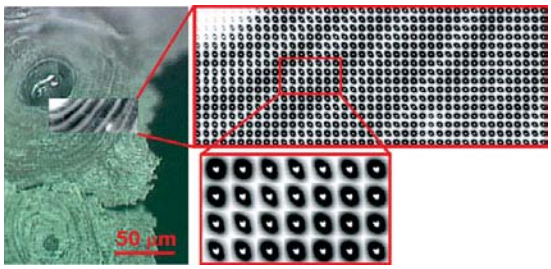

Fig. 2: Image of the integrated SAXS intensity (insert), and of the 2D SAXS patterns from osteonal bone (data taken at ESRF, beamline ID13 with 1 µm beam size from a Kirkpatrick- Baez mirror system).

Closer inspection of the shape of the SAXS patterns shows that the contrast is actually an orientation contrast, arising from an alternating orientation of the mineral platelets with respect to the osteon axis.

Bio Preparation Laboratory

Investigation of soft matter using synchrotron radiation requires adequate sample preparation which is as important as the measuring process itself. The structure of highly hydrated samples such as biological tissues or single cells is strongly altered by dehydration procedures. Moreover, radiation damage due to the high brilliance of synchrotron radiation is one of the most critical issues for biological systems. Therefore, special preparation procedures and measurements under cryo-cooled conditions are frequently necessary, which emphasizes the need for a properly equipped laboratory in the direct neighbourhood of the beamline. A particularly important task is the development of proper sample platforms which allow the transfer of (cryo-cooled) specimens between different instruments, such as the SAXS/WAXS/XRF station, optical microscopes, infrared- or Raman spectrometers, etc.

The planned bio-preparation laboratory in its first construction phase will comprise equipment for conventional cryo-preparation procedures such as freeze drying and critical point drying, and a cryo-microtome to prepare thin sections of tissues. The Lab is presently being built and equipped in a concerted action between our group, BESSY and the University of Heidelberg and should be ready to work in the second half of 2005.

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References:

[1] Jaschouz, D., Paris, O., Roschger, P., Hwang, H-S. and Fratzl, P.: Pole figure analysis of mineral nanoparticle orientation in individual trabecula of human vertebral bone. J. Appl. Cryst. **36**, 494-498 (2003).

[2] Zizak, I., Roschger, P., Paris, O., Misof, B.M., Berzlanovich, A., Bernstorff S., Amenitsch, H., Klaushofer, K. and Fratzl, P.: Characteristics of mineral particles in the human bone-cartilage interface. J. Struct. Biol. **141**, 208-217 (2003).

[3] Gupta, H.S., Misof, K., Zizak, I., Meßmer, P., Roschger, P., Bernstorff, S., Klaushofer, K. and Fratzl, P.: Mineralized microstructure of calcified avian tendons: a scanning small-angle x-ray scattering study. Calcif. Tissue Int. **72**, 567-576 (2003).

[4] Paris, O. and Müller, M.: Scanning X-Ray Microdiffraction of Complex Materials: Diffraction Geometry Considerations. Nucl. Instr. Meth. Phys. Res. B **200**, 390-396 (2003).

[5] Fratzl, P.: Small-angle scattering in materials science – a short review of applications in alloys, ceramics and composite materials. J. Appl. Cryst. **36**, 397-404 (2003).