Research in the Department of Biomaterials is highly interdisciplinary with a focus on biological and bio-inspired materials. Biological materials constitute most of the body of plants and animals around us. They allow cells to function, eyes to capture and interpret light, plants to stand up to the light and animals to move or fly. Biological structures have always been a source of inspiration for solving technical challenges in architecture, mechanical engineering, or materials science. Nature has developed – with comparatively few base substances, mainly polymers and minerals – a range of materials with remarkable functional properties. The key is a complex, often hierarchical structuring of the natural materials.

It is not evident that the lessons learned from biological materials will be applicable immediately to the design of new engineering materials. Indeed, bio-inspiration is not merely the consequence of an observation of naturally occurring structures. These structures are probably good solutions found by a long adaptation process during evolution, but Nature has to take into account a multitude of boundary conditions (mechanical, biological, related to nutrient supply, etc.) which we hardly know and which might not be important in an engineering context. As a consequence, we have to study carefully the biological system and to understand the structure-function relation of the biological material together with its physical and biological constraints, before it may serve as a model for the design of new materials.

With this paradigm in mind, we have defined the research programme of the Department of Biomaterials as a combination of research on natural tissues and on bio-inspired materials. Accordingly, the Department is organised in several research groups, as shown in Fig. 1, and who present their own reports within this volume. In addition, several independent researchers on the postdoctoral level are working on related topics and their reports are summarized jointly in one of the sections (Biological and Bio-inspired Materials).

First, a large effort is devoted to improve our understanding of some biological tissues from a materials science point of view. This requires that we study structure-mechanical function relations, considering the natural environment in which these materials live and grow. One of these tissues is the plant cell wall, a composite of (semi-crystalline) cellulose fibrils in an amorphous polymer matrix. This cell wall material has remarkable mechanical properties which may be tuned by the cell over a wide range of stiffness, according to needs, and which is even capable of generating stresses to provide motility. This is described in the reports by Ingo Burgert and by Rivka Elbaum (independent Humboldt Postdoctoral Fellow).

Mineralized tissues are a second example of mechanically outstanding biological materials. Currently, our emphasis is on elucidating the origin of the fracture resistance of bone, not least because of the biomedical importance of this tissue. Most recently, a hierarchical deformation pattern was discovered as a major reason for the mechanical quality of bone tissue (see report by Himadri S. Gupta). These studies are now being extended to deer antler, which is a rapidly growing bone tissue with even higher toughness than bone. The structural origin of the mechanical performance of teeth and the biomineralization of tooth enamel are being addressed by the postdoctoral researchers Paul Zaslansky and Barbara Aichmayer. Their reports are included in the section “Biological and Bio-inspired Materials”. Finally, collaborative research on the hierarchical structure and the mechanical properties of glass sponge skeletons is also reported in this same section.

It is 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-

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Fig. 1: Research groups in the Department of Biomaterials with respective group leaders

- **Biological and Bio-inspired Materials** (Independent Researchers)
  - **Plant Biomechanics** (Ingo Burgert)
  - **Mechanobiology** (Richard Weinkamer)
  - **Mineralized Tissues** (Himadri S. Gupta)
  - **Synchrotron Diffraction Beamline** (Oskar Paris)
  - **Bone Regeneration** (Manjubala Inderchand)
  - **Bone Material Quality** (Peter Fratzl)
  - **Mesoscale Materials** (Oskar Paris)
repair. These questions are addressed mostly by theoretical means in the research group on mechanobiology (see report by Richard Weinkamer). Moreover, the mechanics of micro-containers and membranes is investigated together with the Interface Department.

In parallel to the study of biological materials, we address topics (right column in Fig. 1) which use the knowledge on biological materials for research either with implications in materials or in biomedical sciences. A major topic is related to bone material quality in osteoporosis and its changes with treatment of the disease. This is a long-term collaboration with the Ludwig Boltzmann Institute of Osteology in Vienna, Austria. Recent results obtained in the last two years relate, for example, to bisphosphonate treatment of osteoporosis and of brittle bone disease (osteogenesis imperfecta), see the section on Bone Material Quality.

A further topic with biomedical implications is bone regeneration. Bone is among the few tissues in our body which are able to heal and to regenerate completely without leaving a scar. In collaboration with the Charité Medical University and other partners in Berlin and Brandenburg, we are now trying to elucidate the healing process in bone, as well as the physical and biochemical factors which govern it. A new Collaborative Research Center (SFB760) supported by the German Science Foundation, and in which the Department of Biomaterials is heavily involved, is starting in the beginning of 2007. The Department is also member of the Berlin Brandenburg Center for Regenerative Therapies (BCRT) supported by the Ministry of Science (BMBF). Current research is centred on the analysis of the various tissues occurring during bone healing, as well as cell and tissue growth on porous scaffolds, which might serve as implants; see the report by Manjubala Inderchand.

Biomimetic materials are currently developed in one of the research groups, based on thermal and chemical processing of plants. In particular, the processing is studied in detail by in-situ synchrotron diffraction. Further research in this group concerns the behaviour of fluids in mesoporous materials, which are studied in collaboration with partners in Berlin within the framework of the Collaborative Research Center SFB448 (see report by Oskar Paris). Work on bio-inspired active hybrid materials based on gels and microstructured silicon is also conducted in the Department in collaboration with Bell Labs, USA (see section on Biological and Bio-inspired Materials).

Finally, a large effort is devoted to establish new methods for analysis of biomaterials. Indeed, studying hierarchical biomaterials requires state-of-the-art experimental equipment and there is also some need for the development of new approaches. One strategy is to set up a suite of scanning imaging methods which may be applied to the same specimen and which give different type of information about the material with a position resolution in the micron range. We are currently using scanning electron microscopy and scanning x-ray diffraction to characterize the micro- and nanostructure. Moreover, we have established Raman imaging to provide information on chemical composition and nano-indentation as well as acoustic microscopy to estimate local mechanical properties. The strength of this multi-method approach is that the different parameters measured on the same specimen can be correlated at the local level. This helps finding structure-property relations even in extremely heterogeneous materials. In-situ techniques are a second type of approach, where 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 the process itself. In some cases, we can perform such studies in the laboratory (e.g. with Raman or infrared spectroscopy or in the environmental scanning electron microscope), but in many cases we need synchrotron radiation (e. g. for x-ray diffraction or small-angle scattering). A large project in this context is the setting up of a dedicated scanning small- and wide-angle scattering beamline at the synchrotron BESSY in Berlin. The end station for this beamline has been developed under the supervision of Oskar Paris (see his report) and is now performing the first user experiments.

Peter Fratzl
Director of the Department of Biomaterials

BIOLOGICAL MATERIALS

Plant Systems Biomechanics

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. It is also a valuable source for extracting biomimetic principles for the design of new bio-inspired materials [Fig. 1].

Plants are hierarchically organized which means that their macroscopic properties mainly originate from the nanoscale. Nanometer thick semi-crystalline cellulose fibrils embedded in amorphous matrix polymers are the basic assembly of plant cell wall structure. But at these levels of hierarchy, plants manifest a wide variety of adaptable parameters such as cell shape, thickness and arrangement of cell wall layers, the orientation of cellulose microfibrils within cell walls, and in the chemical composition of individual cell wall layers. Micromechanical approaches are well suited to characterize these composite structure for understanding both the material design and the optimization strategies of living plants [1].

Deformation mechanisms in secondary cell walls were examined with various in-situ techniques which simultaneously combine micromechanical tests with (nano-)structural analysis [3, 4]. Tensile tests on mechanically isolated cells [5] combined with simultaneous acquisition of Raman spectra have shown that the (stiff) cellulose fibrils carry most of the load with only small and fully elastic deformation [3]. Almost all of the deformation takes place by shear of the (deformable) hemicellulose/lignin matrix. This combination confers both stiffness and toughness to the cell wall.

Enzymatic treatments were utilized to suppress the functioning of individual polymers in the mature cell wall of secondary xylem cells. Micromechanical tests on the modified material revealed the mechanical relevance of hemicelluloses (xylan) in the composite structure.
Stress Generation and Plant Movement

Active movement is usually associated with animals rather than plants. Clearly, plants do not have muscles but they are able to pre-stress their tissues in order to actuate their organs. We showed for normal wood and compression wood of spruce (Picea abies) that either tensile or compressive stresses can be obtained during swelling of the cell wall, depending on the ability of the cell to undergo some torsion [6]. This was shown in swelling experiments on individual cells and tissues and can be well understood by simple mechanical considerations taking into account the cell shapes and the observed cellulose fibril orientations (Fig. 3).

The almost inextensible cellulose fibrils redirect the forces generated by the swelling of the matrix by purely geometrical considerations taking into account the cell shapes and the observed cellulose fibril orientations (Fig. 3).

Bio-Inspired Materials

A) Gradients in Plants

Palm trees have evolved gradual transitions between stiff, sclerenchymatous supporting fibres and soft parenchymatous tissue, functioning as a matrix. This structure can help to avoid critical shear stresses and separation of the material at the interfaces when plants are mechanically loaded. Here, we investigated in cooperation with the University of Freiburg (Lab. T. Speck) gradual transitions in arborescent palms at different hierarchical levels by anatomical, micromechanical, physical and biochemical methods taking Washingtonia robusta as a model organism [Fig. 4].

The stress-strain curves show a change in cell wall stiffness from the centre to the middle of the fibre cap. The underlying structural and biochemical features are currently studied. Our aim is to transfer the concept of gradual transitions into technical application for innovative structurally optimised composite materials.

Bio-Inspired Materials

B) Fibre-Matrix Interactions

The nanocomposite structure of the plant cell wall with its specific interface design between stiff cellulose fibrils and plant matrix polymers can be taken as a source of inspiration for a transfer to technical applications. Currently we are running a cooperation project (partners: University of Freiburg (Lab. T. Speck) and ITV Denkendorf) in the framework of the BMBF-Bionik competition on an improvement of technical composites with a new concept for the embedding of glass fibres based on the primary cell wall assembly. With the aid of our mechanical workshop (G. Haseloff) we developed embedding and pull-out test setups to mechanically characterize the interface properties between fibre and matrix.

References:


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BIOLOGICAL MATERIALS

Bone Material Quality and Osteoporosis Research

Bone is a hierarchically structured material with remarkable mechanical performance. Understanding its properties is essential for the assessment of diseases such as osteoporosis, for a critical evaluation of current therapies and to aid in their more targeted development. While the full hierarchical structure of bone is extremely complex and variable, its basic building block, the mineralized collagen fibril, is rather universal. The mechanical performance of bone, often coined “bone quality” [1], does not only depend on the shape and the amount of the bone (as estimated by the bone mineral density, BMD), but also on its architecture and on the quality of the bone material. Current research carried out primarily in collaboration with the Ludwig Boltzmann Institute of Osteology (Vienna, Austria) concentrates on studying the structural basis of bone material quality and changes due to disease or treatment.

Anisotropy of Fracture Toughness in Human Compact Bone

Bone material quality depends to a large extent on the orientation of collagen fibrils in bone tissue. In collaboration with H. Peterlik (University of Vienna), we studied controlled crack extension in human femur [2]. It was shown that the energy dissipated by the crack is two orders of magnitudes larger if it propagates perpendicularly to the collagen fibrils than when it runs along them. The reason is obvious in Fig. 1. When the crack follows the main collagen direction, it runs straight and the dissipated energy is low (Fig. 1B). In contrast, the crack path is zigzagging when it should run perpendicularly to the collagen direction (Fig. 1A), thus dissipating much more energy.

Mineral Density in Different Bone Matrices

Since mineral is the stiffer component in bone, it is not surprising that the elastic modulus of the bone matrix depends on the mineral content. Fig. 2 shows that this dependence is not linear. Even more important, the relation between local elastic modulus (as measured by nanoindentation) and local mineral content (as measured by backscattered electron imaging) depends on the type of organic matrix, for example when one moves from bone into mineralized cartilage in a joint [3].

Raman Imaging of Bone

A considerable effort was undertaken by Murat Kazanci (postdoc) to establish Raman imaging as a tool for studying bone material quality. The reason for the interest in this technique is that it allows the imaging of material parameters with one micron spatial resolution and spectral resolution much better than infrared spectroscopy. The Raman signal from compact bone turned out to be extremely sensitive to tissue orientation (Fig. 4) and methodology was devised to

Fig. 1: Crack propagation in a human femur, perpendicular to the long bone axis (A) and parallel to it (B), from [2].

Fig. 2: Dependence of indentation modulus on mineral content in mineralized cartilage. The lines correspond to a composite model [2].

Fig. 3: Histogram of mineral density distribution in trabecular bone matrix of biopsies from patients with a polymorphism in the COL1A1 gene [3].

References:

Further reading:
[2] Our analysis showed significant reduction in matrix mineralization in bone biopsies from heterozygotes compared with homozygotes [see Fig. 3].


[5] Since 1993: Visiting Professor, (Physics Department of the University of Munich).


[8] Since 2004: Honorary Professor of Physics at Humboldt University Berlin.

[9] Since 2006: Director, Department of Biomaterials (Max Planck Institute of Colloid and Interfaces, Potsdam).
Bisphosphonate Treatment of Brittle Bone Disease

Brittle bone disease (osteogenesis imperfecta, OI) is a disorder which is linked to genetic modifications of the collagen gene and which leads to enhanced bone fragility. Children with this disease suffer from multiple fractures and associated complications. It is known that the fracture incidence in patients can be reduced by a treatment with bisphosphonates. It is not known, however, in which way this treatment affects the bone quality and leads to a reduction of bone fragility. In collaboration with the Hospital for Special Surgery (New York, USA), we studied a mouse model of this disease treated with the bisphosphonate alendronate (ALN). It was found that ALN augmented the mechanical, geometrical, and material properties of cortical and trabecular bone in controls, while the only observable improvement to the OI mouse model was increased bone volume [9], see Fig. 5.

This work was continued by studying biopsies from young OI patients treated with the bisphosphonate pamidronate (PAM), known to induce bone formation, combined with osteoprotegerin (OPG), known to act on bone resorption, was explored [10]. While the OI bone tissue was found stiffer and more mineralized than controls, the anti-fracture effectiveness of PAM treatment was primarily due to an increase of bone volume (see Fig. 6). This result is very similar to what was found for the animal model.

BIOLOGICAL MATERIALS

Mineralized Tissues

Our research looks at the structural adaptation of mineralized tissues to their mechanical function at the length scale of a micron and below. At this level, the extracellular connective matrix in both vertebrate and invertebrate organisms often consists at the molecular level of a composite where organic molecules (such as collagen or chitin) are inter-penetrated with inorganic crystallites (typically calcium phosphates or carbonates) to form an anisotropic, hard and tough material. Weight for weight, such biomineralized tissues compare favorably with man-made composites, although requiring much lower temperatures and processing conditions. Therefore, an understanding of the structural design principles in such biomaterials may provide guidelines in making new strong composite materials. In addition, understanding how perturbations in the mineralized microstructure affect mechanics (in bone diseases like osteoporosis) would be important in developing treatments for such pathological conditions.

Bone consists of a compact tissue type (cortical bone) and a spongy, porous material (trabecular bone). In both tissue types, the basic building block is the bone lamella, typically about 5 µm thick. In cortical bone, lamellae form laminated cylindrical composite structures built around blood vessels, which are denoted as secondary osteons. While understanding the internal architecture of such osteons is crucially important for bone biomechanics, a convincing and quantitative structural model has thus far been elusive. Using a novel combination of high brilliance synchrotron radiation with a micron-sized beam and local crystallographic texture measurements [1], we were able to show that the collagen fibers are arranged in layers of varying helical pitch with respect to the osteon long axis, in effect forming a right handed spiral motif (Fig. 1) [2]. Such a spring like structure would be capable of absorbing elastic energy during physiological motion, and may act as a buffer preventing microcracks from penetrating to and destroying the sensitive inner blood vessel.

Using a combination of scanning microprobe methods (nanoindentation and backscattered electron microscopy) we were able to further elucidate the mechanical structure of the osteon. We showed that it consists of layers of alternating high and low stiffness within a single lamella and that the layers of lower modulus correspond to regions of lower average mineral content [3]. Such a compositionally and mechanically modulated structure is an example of a functionally graded material, and would act as an excellent crack stopper, as has been demonstrated theoretically.

The bone lamellae are comprised of mineralized collagen fibrils, which are 100 to 200 nm diameter composites of type I collagen and hydroxyapatite mineral embedded in a small amount of extrafibrillar matrix. Using in-situ mechanical testing with time-resolved synchrotron X-ray diffraction, we showed that the fibrils in bone take about half the total deformation in the tissue under tensile load, and do not stretch further in the inelastic regime. Based on these findings, we proposed a fibril level model of interfibrillar shearing, where the total strain is divided into a tensile component carried by the fibrils and shearing in the thin layers of extrafibrillar matrix (~ a few nm thick) between them (Fig. 2) [4]. Above the mechanical yield point, a stick-slip mechanism of interfibrillar sliding results [5], which leads to a large work of fracture.

To understand the way strain is transferred down the structural hierarchy, a novel combination of tensile testing of single fibrolamellar bone packets with wide-angle synchrotron X-ray diffraction and small angle X-ray scattering was used. This technique enables us to measure, concurrently, the strain in the tissue, the fibrils and the mineral particles. UV-laser microdissection enabled the isolation of single bone packets at the tissue level, excluding all structures at higher length scales in the hierarchy. Strain is passed down in successively lower fractions from the tissue down to the molecular level (Fig. 3) [6], via shearing strains in the intervening extra- and interfibrillar organic matrix, and depends on the degree of hydration of the organic matrix. Such an arrangement results in a high stiffness of the overall material while protecting the brittle hydroxyapatite phase from excessive load.
Using a thermally activated stress flow analysis originally developed to study plastic deformation in metals, we established that the fundamental molecular step in plastic deformation of bone takes place in a volume of about 1 nm$^3$, and requires activation energy of about 1 eV. Based on the magnitude of these quantities, a model for bone fracture was proposed, where breakage of ionic bonds (in the extrafibrillar matrix) between long irregular polyelectrolyte chains and divalent ions like calcium mediate bone plasticity (Fig. 4) [7]. Modifying or altering the properties of this extrafibrillar “glue” could be an effective way to tune the properties of bone, and is a current focus of our research.

References:
BIOLOGICAL MATERIALS

Bone Regeneration

Our group deals with two different approaches on similar themes of bone and tissue regeneration. The first aim is to understand the processes underlying the new bone tissue formation intrinsically both by physical and biological approaches. A biomaterial scaffold is used as a template to analyze the behavior of the pre-osteoblastic bone cells to produce new bone-like tissue.

The second aim is to acquire more knowledge on the properties of the tissues formed during bone healing process which could lead to understand the mechano-regulation of the biological process during fracture healing, by the application of our multi-method approach. Further this multi-method approach is applied to study the quality of the bone material in bone biopsies related to bone diseases and their treatment. The project of fracture healing has started this year within the framework of Sonderforschungsbereich (SFB) 760 focused in Berlin with research partners from Charité-Universitätsmedizin Berlin, GKSS Institute for Polymer Research at Teltow.

New Bone Tissue Formation in-vitro

(A) Bone Replacement Scaffolds via Rapid Prototyping

An ideal scaffolding material for bone tissue engineering should replicate the bone anatomy at microscopic level with interconnected micro and macro pores and with a similar composition of nanocomposite and should be able to promote the osteoblast proliferation and expression of the osteoblastic phenotype. Solid freeform fabrication or rapid prototyping (RP) is a technology by which a complex three dimensional (3D) structures can be produced directly from computer generated (CAD) design. CAD and RP together can be used to control the macro and micro-architecture of porous scaffolds. Two different types of rapid prototyping methods were used suitable to produce ceramic and polymer composite scaffolds respectively. A rapid prototyping system based on “Digital Light Processing” called Envisiontec Perfactory Mini (Envisiontec, Germany), was used for resin molds and a 3D wax printer, Solid scape Model maker II (Solid scape, USA) was used to produce wax molds. We aim at developing a hydroxyapatite and a polymeric composite scaffold with defined internal architecture by RP method. Sintered dense hydroxyapatite scaffolds were produced using resin molds by slurry casting method and characterized [1,2]. To mimic the components of bone, a biopolymer such as chitosan is used in combination with apatite to form a composite scaffold. Chitosan has been proposed to serve as a non-protein matrix for three-dimensional tissue growth, a potential candidate for tissue engineering and drug delivery systems. The composite scaffolds are produced using dissolvable wax moulds and then freeze dried and cross-linked to produce micro pores to enhance vascularisation in the scaffolds [3].

Cell attachment, proliferation and differentiation over time on a material are indication of cellular compatibility of the material and determine the suitability of the material for tissue engineering application. The fabricated hydroxyapatite and chitosan/apatite scaffolds were accessed for their biocompatibility with bone cells using pre-osteoblastic cell line. The cells cultured on scaffolds proliferated over the material and pores in multilayer and produced extra-cellular matrix in 3 weeks, as seen from histological staining (Fig. 1). The structure of the scaffold allows more cells to grow compared to two-dimensional matrices [3].

Additionally, the effect of additional factors such as osteogenic hormones and growth factors on the proliferation and differentiation of the cells in scaffolds are investigated [4].

(B) Tissue Growth on Biomaterials of Controlled Geometry and Stiffness

Bone regeneration is influenced by biochemical, biomechanical as well as cellular mechanisms. On the level of single cells, it is well investigated that initial cell attachment and following cell spreading and proliferation is determined by surface topography at the nano- and micrometer scale. But beyond those levels, cells have developed highly sophisticated and active mechanisms to probe their environment. Physical parameters of supports, such as scaffolds, may also have an impact on cell amplification and furthermore, on tissue formation.

For this purpose we established a model system, which allowed in parallel microscopic observation as well as quantification of new tissue formation in a three-dimensional environment. We used three-dimensional hydroxyapatite plates containing channels of various shapes (triangular, squared, hexagonal and round) and three various sizes. These hydroxyapatite plates were produced via rapid prototyping method mentioned earlier. Tissue formation occurs in that way, that, independent form the original shape, the new formed tissue keeps a round central canal.

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Fig. 2: Extracellular matrix (ECM) tissue growth in 3D channels of various shapes showing that the growth is independent of shape and forms a round central channel.

This amplification modus is maintained throughout the whole tissue into the depth of the channels, observed with confocal laser scanning microscopy [Fig 2]. Following 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. In that process the development of mechanical forces within the tissue itself may play a key role in growth behavior. Thus, tissue formation in vitro is also determined by physical properties. Additional to the native conditions, nanomodifications of the surface with proteins, which enhance attachment and are involved in the differentiation process of osteoblasts, especially RGD-peptides, will be used to guide the differentiation of osteoblasts.

Apart from investigating tissue formation in 3D matrices, we also studied the role of individual components of the cells during differentiation and proliferation by physical methods such as X-ray scattering and Fourier transform infrared microscopy and spectroscopy.

Characterisation of Bone Healing and Bone Regeneration Processes

Bone healing is a complex process in which different types of tissue are being formed and remodeled. While the pathologic evaluations describe the spatial and temporal distribution of the various tissue types comprising the callus [Fig 3], little is known of their material properties. In addition, the patterns of appearance of these tissue types as well as their physical properties depend both on biological factors and physical influences, such as mechanical stress. A better understanding of the mechano-regulation of the biological processes during healing requires more knowledge on the properties of the tissues making up the callus. We investigate the spatial distribution and temporal sequence of ultrastructure and mechanical properties of callus tissues over the course of bone healing [5]. We apply 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, Raman imaging, nanoindentation and acoustic microscopy.

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. This project is in close conjunction with the researchers at Charité-Universitätsmedizin Berlin, where the bone healing experiments is carried out in both small and large animal models, as it is known that the tissue architecture is quite different in different animal species.

Fig. 3: The various tissues formed during fracture healing identified by histology. The material properties of these tissues are still unknown.

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 osteoalthritis. The methodologies used are quantitative back-scattered electron imaging (qBEI), scanning nanoindentation and small-angle x-ray scattering techniques.

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Mechanobiology

Mechanical forces play a crucial role for the performance of biological and chemical systems. Mechanobiology studies how mechanical forces control the development and maintenance of living tissues and how their structure adapts to changes in the mechanical environment. Computational approaches have proven successful in gaining insight into the relation between local rules describing the action of living cells and global changes in the structure. The aim of our work on the mechanobiological system of trabecular bone is to understand the relation between the processes of remodeling and mineralization and its effect on the structure at two different hierarchical levels: trabecular architecture and bone material.

The applicability of man-made micro-capsules depends strongly on a control of their mechanical properties. We have studied the interplay between mechanics and chemistry for capsule systems made of catanionic amphiphilic molecules and polyelectrolytes.

Trabecular Bone: Architecture

Living trabecular bone is continuously remodeled by the resorption and deposition of bone packets. The probability for deposition is increased (decreased) at sites with a high (low) mechanical loading. A crucial unknown for a deeper understanding of the remodeling process is the phenomenological remodeling rule at the core of the controlling feedback loop. It relates the local mechanical stimulus to the probability for bone resorption/deposition at the bone surface. We developed a computer model, which allows the implementation of different remodeling rules, and studied their effect on the trabecular architecture and its time evolution. In our simulations we found features that are independent of the remodeling rule, e.g., the emergence of a network-like structure and the coarsening of the structure by a reduction of the number of trabeculae and thickening of the remaining ones, while the bone volume fraction remained constant. Strongly dependent on the remodeling rule are architectural parameters like the bone surface roughness, the velocity with which coarsening of the structure proceeds and the response of the system to external perturbations. An example is given in Fig. 1, where the probability for bone resorption was varied, a parameter accessible by present-day medications. Depending on the implemented remodeling rule, this variation has either almost no or a significant effect on the resulting bone volume fraction. Comparison with real bone lead us to the conclusion that in real bone a remodeling rule with an activation threshold for the bone depositing cells similarly to the step-remodeling rule of Fig. 1 is active.

Trabecular Bone: Material

At the material level, trabecular bone consists of a patchwork of bone packets with different mineral content. This structure is the result of remodelling and a process of mineralization, which leads to a temporal increase of the mineral content in the initially unmineralized bone packet. The heterogeneity of the mineral content is usually characterized by a frequency distribution, the bone mineralization density distribution (BMDD). For healthy humans, experiments demonstrated that the bell-shaped BMDD is almost unchanged during life time. With a theoretical model, which considers both processes, remodeling and mineralization, we could connect the shape of the BMDD with the mineralization law, which describes the increase of the mineral content with time in a single bone packet. For the mineralization law in healthy humans our model predicts a rapid increase in the mineral content up to more than 50% of the total capacity followed by a much slower phase which extends over several years. An important application of the model is to predict the time evolution of the BMDD due to changes in the turnover. Some bone diseases, the most prominent being osteoporosis, are connected with an increased turnover, while standard medications try to reduce the bone turnover. A simulated therapy of a high-turnover osteoporosis with turnover-reducing drugs showed that transiently the mineral content displays an extraordinary homogeneity.

Fig. 1: Comparison between two different remodeling rules for bone remodeling: linear, which assumes a linear relationship between mechanical stimulus and bone deposition probability and step, which assumes a step function for this relation. Some changes in the activity of bone resorbing cells for a linear remodeling rule with different slopes and a step-remodeling rule with different step position are shown in the plot.
design patient-specific therapies which bring an abnormal BMDD back to its original healthy state.

Fig. 2: The frequency distribution of the mineral content in bone (BMDD) and its time evolution during an antiresorptive therapy. Starting from a BMDD of increased turnover (red) and reducing the turnover to normal levels, brings the BMDD in the long term back to its healthy distribution (blue). Transiently (3 years after the start of the therapy) the BMDD displays a sharp peak (green).

Catanionic Bilayers

Catanionic systems are mixtures of amphiphilic molecules with oppositely charged headgroups. Arranged in bilayer structures the electrostatic forces result in an increase of the lateral cohesion energy. Peculiar mechanical behavior was observed experimentally, for example the formation of faceted hollow polyhedrons [6] and the extreme sensitivity of the phase diagram with respect to the molar ratio between anionic and cationic surfactants. Using computer simulations we explored the mechanical properties of a model membrane.

On a mesoscopic scale the model membrane consists of an apolar core and an upper and lower charged layer formed by the headgroups of the molecules [Fig. 3]. In the microscopic description of the charged layer the headgroups occupy a triangular lattice. Two types of interaction are considered: the electrostatic interaction between headgroups and the hydrogen bonds between neighboring anionic headgroups modeled by harmonic springs. Membranes with a varying composition of anionic and cationic molecules have been first thermodynamically equilibrated and then mechanically tested. In agreement with experimental observations the simulation showed for high anionic concentrations extremely large bending rigidities of \( \kappa > 500 \ k_B T \) [Fig. 4]. This stiffening of the membrane results from a rigidity percolation, i.e., the formation of a rigid backbone of hydrogen bonds in the charged layer. The mesoscopic sandwich-like structure of the membrane amplifies this effect since the apolar core separating the charged layers acts via a kind of lever-arm principle. Striking is also the narrowness of the region of concentrations in which the transition between soft and stiff bilayers occurs. In the case of electrostatic ordering between the molecules, the stiffening transition is postponed to higher concentrations of anionic headgroups further sharpening the soft-to-stiff transition.

Fig. 3: Cross-section through a lipid bilayer membrane with negatively (black) and positively (yellow) charged headgroups. The schematic black bars connecting two neighboring anionic molecules indicate the formation of additional bonds (e.g., hydrogen bonds). The two charged bilayers are separated by an apolar core of low bending rigidity \( \kappa_0 \).

Polyelectrolyte Capsules

In collaboration with the Department of Interfaces we analyzed the deformation data of polyelectrolyte micro-capsules of well-defined geometry obtained with the atomic force microscope. In the limit of small deformations analytical results of shell theory can be applied, which were complemented by finite element calculations, giving quantitative information about the elastic modulus of the capsule wall material [7]. Variations of temperature and salt concentration lead to changes in the mechanical properties of the wall material and to changes in the capsule diameter [8,9]. The swelling-to-shrinking transitions were explained by an interplay of an expanding electrostatic force and a contracting surface tension [9].

References:


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This section reviews some of the work on biological and bio-inspired materials conducted outside the research groups either with external partners or by postdoctoral researchers working independently (B. Aichmayer, R. Elbaum and P. Zaslansky).

Structure and Properties of Glass Sponges

The structure and the mechanical design of different glass sponges are investigated in collaboration with colleagues from Bell Labs (Joanna Aizenberg and Co-workers), UCSB (James Weaver and Dan Morse), among others. A major result is the description of the hierarchical structure of the glass sponge Euplectella [1], consisting of glass spicules joined by a silica matrix (Fig.1). The spicules consist of laminated glass with a succession of micron-sized silica layers and nanometer-sized protein layers and possess remarkable mechanical properties [2].

Mechanics and Thermodynamics of New Materials

A further collaboration with Bell Labs (J. Aizenberg and co-workers) is the development and mechanical description of new types of active materials based on hydrogels stabilized by silica posts which may be free-standing or attached to a surface (Fig. 2). The silica posts bend reversibly upon drying of the gel, leading to the formation of complex micro-patterns and to micro-actuation [3]. Further research with the University Leoben includes, for example, the theoretical description of unstable and moving interfaces in materials [4], or the fracture mechanisms in certain polymers at the micro- and nanoscale [5].

Bio-Inspired Polymer-Mineral Composites (Barbara Aichmayer)

In biomineralization, the nucleation and growth of inorganic crystals are controlled by biological macromolecules. For instance, amelogenin proteins play a key role in the formation of tooth enamel. In cooperation with H. Margolis et al. (Forsyth Institute, Boston) and R. Sigel (Colloid Dept.) we studied the self-assembly of different amelogenins in solution [6]. It was shown that amelogenins form so-called "nanospheres", which can further aggregate into assemblies of multiple nanospheres (see Fig. 3).

Further studies showed that the shape of the individual building blocks can better be described by an ellipsoidal (or even disc-like) shape and that the aggregation depends more strongly on the pH value than on the temperature. In the presence of growing elongated hydroxyapatite crystals, the aggregates sketched in Fig. 3 might be modified towards a higher degree of ordering, which would imply a parallel alignment of the mineral crystals, as typical for the structure of enamel. In order to elucidate, how the protein assemblies guide the crystal growth in enamel formation, our current and future research activities focus on in vitro mineralization studies.

Biomineralization can be mimicked by using artificial polymers to manipulate crystal growth. In cooperation with H. Colin et al. (Colloid Dept.), we investigate the biomimetic formation of calcite crystals in the presence of polysynthetic sulfonate [7]. Scattering measurements of single mineral particles, using a μ-focus beam (at BESSY, Berlin and ESRF, Grenoble) were performed to study the influence of PSS on the structure of micron-sized calcite particles. First results show that the polymeric additive led to a transition from single crystals to strongly textured polycrystals with structural features below 10nm.

Finally, the nucleation and growth of metal nanoparticles on bacterial S-layers were investigated quantitatively by small-angle x-ray scattering [8].

The general scope of these structural studies is to contribute to a better understanding of biological and biomimetic mineralization.

The Materials Design of Wheat Awns for Seed Dispersal (Rivka Elbaum)

Awns evolved to direct seeds to a safe germination position. The dispersal unit of wild wheat (Triticum dicoccoides) bears two pronounced awns that balance the seed as it falls to the ground [9].
Using X-ray diffraction we found that the cellulose fibrils, which construct the cell walls, are aligned mostly along the long axis of the awn, except at a region close to the seeds (highlighted in Fig. 4). In this location the fibers are randomly oriented. This design results in bending of the awns with the long axis of the awn, except at a region close to the seeds which construct the cell walls, are aligned mostly along the right, and the active cellulose zone is indicated in pink.

Cycling the air humidity causes a periodic movement of the awns, resembling the swimming motion of frog legs. Fig. 5 shows this principle of the dispersal unit movement. It is clearly visible that the average distance between the awns changes as a function of air humidity. With the daily humidity cycle, the awns will move cyclically and thereby propel the dispersal unit forward. Silicified hairs that cover the awns are randomly oriented. This part of the awn will expand in all directions, pushing the awns toward each other. With drying, this active region will contract, similarly to a muscle.

Much of our 3D characterization is based on imaging of interference patterns and as a result we obtain spatial sub-micron resolution using currently available partially-coherent laser and X-ray sources [12]. As seen in Fig 6, our methods produce data with a resolution capable of resolving ~1 micrometer thick tubules in dentin (or similarly prisms in enamel). We are thus able to track displacements and image the microstructure, and our efforts are aimed at correlating the deformation patterns so as to understand the behavior of human teeth under physiological (daily) mechanical load.

Fig. 4: A graphic illustration of the wild wheat plant and two dispersal units (not in scale), are shown on the left. Each dispersal unit carries two pronounced awns that orient the dispersal unit as it falls. The red square indicates the location of the scanning electron micrograph on the right, and the active cellulose zone is indicated in pink.

Fig. 5: Wild wheat awns at different levels of relative air humidity (r.h.)

Structure-Function Relations of Human Teeth in 3D (Paul Zaslansky)

Teeth are composed of mainly two carbonated hydroxyapatite based composite materials (enamel and dentin), arranged in a complex array of graded and varying micro structures. Systematic structural variations of tooth materials [11] lead to very different responses to load within different parts of the crown and root. Consequently, the function of whole teeth and the nature of differences between different types of teeth are not well understood. Our work focuses on trying to better understand design principles of human teeth by combining 2D imaging techniques (wet-mode environmental scanning electron microscopy, X-ray scattering techniques and Acoustic and Raman microscopy) of static and mechanically loaded tooth samples with 3D high resolution (sub µ) measurements (using microtomography and speckle interferometry). Our working premise is that there is great importance to both arrangement and properties of features embedded in the microstructure [11,12] and both are needed to support the longevity of teeth. By matching structural and deformation patterns from 2D slices with non-destructive measurements of the 3D samples, we hope to understand what allows teeth to function without remodeling or ‘self-repair’ the way that bones do.

Fig. 6: 3D virtual cube of dentin displaying tubules running upward through the structure. The phase enhanced image, obtained at ID19 in the ESRF clearly shows the distribution, density and orientations of the highly mineralized tubules. Scale bar: 100 µm

References:
Mesoscale materials exhibit particular structural features at intermediate scales between the atomic/molecular world and macroscopic dimensions. Such systems may show novel properties and functions which result directly from the size of the compartments and/or the interactions between the individual structural units. Our research is directed towards the structural characterization and the understanding of structure-function relationships of hierarchical mesoscale composites such as biological materials, and [biomimetic] carbons and ceramics. Moreover, we are interested in the phase behavior of fluids in confined geometry of mesopores, and in their elastic interaction with the solid pore walls. Our experimental approaches are essentially based on scattering techniques using synchrotron radiation. We develop sophisticated new in-situ methods to "watch materials at work", and we apply microbeam scanning techniques to map the local nanostructure in hierarchically organized materials.

**Biomimetic Processing**

The aim here is to transform hierarchical plant tissues into inorganic materials, and to characterize their structure and transformation behavior. In a first approach, infiltration of wood with ceria nanoparticles in a suitable acidic solution was successfully used to replace the lignin phase with the inorganic materials, and to characterize their structure and mechanical properties. Differences between different carbon fiber types were attributed to different amounts of inter- and intracrystalline porosity.

Fig. 1 represents the simple and economical synthesis of novel ceramics with wood as a carbon source. We studied the structural and chemical development of pyrolysed wood up to 2400°C. We could show that crack free carbon monoliths which fully resemble the honeycomb-like cellular architecture of wood tissue, could be produced with a preferred carbon orientation along the cell axis [2]. The thermal decomposition of wood cellulose was further investigated with in-situ X-ray diffraction. By quantitatively analyzing the kinetics for different temperatures it was found that decomposition of the crystalline cellulose in wood occurred mainly via a thermally activated decrease of the microfibril diameter. As a second model system for direct biomimetic conversion of plants, we have investigated the silica accumulating stalks of Equisetum hyemale (horsetail or scouring rush). Besides a general interest in the function of silica in higher plants, we used horsetail for direct SiC synthesis by controlled pyrolysis taking the biopolymers as a carbon source and silica as a Si-source. Ongoing work is focusing on the detailed characterization of the type and distribution of silica, and on the optimization of the conversion process in terms of yield and type of SiC.

**Mesoscale Carbons**

The detailed origin of the extraordinary mechanical properties of carbon fibers and the relation to their local mesoscale structure are still largely unknown. In a pioneering experiment in cooperation with the University of Vienna we combined in-situ bending of single carbon fibers with high resolution X-ray diffraction by scanning the bent fibers across a 0.1 μm wide beam (Fig. 2). We developed in-situ bending to determine the local mechanical properties of carbon fibers.

Fig. 2: Sketch of the in-situ bending experiment to determine the local mechanical properties of carbon fibers.

Further work on mesoscale carbons included the local mechanical properties of pyrolysed wood at the level of single cell walls using nanoindentation [4], and a critical examination of the classical way to obtain carbon crystallite sizes from Raman band intensity ratios [5]. One of the future challenges of our research in this field is related to the important role of covalent cross-links for the mechanical behavior in disordered carbons. In this respect, we have already started in-situ high temperature creep studies of single carbon fibers within the synchrotron radiation X-ray microbeam at the μ-Spot beamline at BESSY.
Fluids in Mesopores

Mesoporous materials with narrow distributions of pores on highly ordered lattices are ideal model systems to study the phase behavior of fluids in confinement. In cooperation with the Technical University of Berlin, we have developed a sorption device for in-situ small-angle X-ray diffraction. Sorption of organic fluids in 2D hexagonal lattices of SBA-15 silica materials was investigated at Hamburger Synchrotronstrahlungslabor (HASYLAB), and more recently at the µ-Spot beamline at BESSY. Analyzing the intensity of the diffraction peaks as a function of vapor pressure along a sorption isotherm provides detailed structural information on liquid film formation and on pore condensation [6]. Moreover, high capillary pressures lead to a deformation of the pore walls at capillary condensation. The adsorption strains related to the deformation of the pore lattice can directly be obtained from the shift of the diffraction peaks as a function of vapor pressure [Fig. 3] [7].

In the future, we propose to investigate the dynamic interaction between pore deformation as a consequence of capillary condensation, and the influence of these deformations on the phase behavior of the fluid. Moreover, we plan to develop a mechanical model that quantitatively explains the experimentally observed behavior. This activity is embedded in the framework of the Collaborative Research Center Sfb 448 “Mesoscopically Organized Composites” of the German Research Foundation (DFG).

From Diffraction to Imaging

A large part of our activities in the field of synchrotron research in the last two years were related to the commissioning of an experimental station for simultaneous microbeam small- and wide-angle scattering (SAXS/WAXS) at the microfocus (µ-Spot) beamline at BESSY in Berlin. The instrument is now fully operational and provides a routine microbeam of 10 μm diameter at a flux of more than 1010 photons per second [8]. A series of user experiments have already been performed in cooperation with other research groups from the department and with external partners. Many of them were related to scanning SAXS/WAXS studies on biological or bio-inspired materials such as for instance bone, plant tissues, and biomimetic calcite.

A long-term goal of our research is to proceed from microbeam scanning SAXS/WAXS to a real imaging technique. There has been considerable progress in our group concerning software development for interactive instrument control combined with online data analysis and online parameter imaging. This work was done in close collaboration with the European Synchrotron Radiation Facility (ESRF) in the framework of a long-term proposal. At present, several software tools are available that allow an automated extraction and imaging of nanostuctural parameters such as local fiber orientation from WAXS [Fig. 4] or particle size and orientation from SAXS. Future work is directed towards the implementation of these tools into the beamline software at BESSY.

Furthermore, we plan to extend our scanning approach to the third dimension in both, the reciprocal space (local texture analysis) and in real space (SAXS tomography, confocal diffraction).

Fig. 3: In-situ sorption of perfluoropentane (CF₅) in SBA-15 (red: adsorption; blue: desorption). a) shows the integrated intensity of the 10 reflection which is low for entirely filled pores due to partial contrast matching, and b) shows the corresponding lattice strains.

Fig. 4: SEM image (left) and an image based on scanning microbeam diffraction (right) of a flow sensing system in cricket appendices. The color scale on the right is given by the intensity of the equatorial 040 reflection from the crystalline chitin fibers in this chitin/protein nanocomposite, and the bars indicate the local chitin fiber orientation as deduced from the individual diffraction patterns. The sample was prepared by using a UV microlaser instrument and the diffraction experiments were performed at the ESRF [9].


References:


