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Bone & Soft tissue Biomaterial interactions

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Intramedullary fixation by resorbable rods in a comminuted phalangeal fracture model: a biomechanical study

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The mechanical rigidity of three different methods of resorbable intramedullary fixation (bone peg, AND polyglycolide rods with and without interlocking) was assessed in a comminuted phalangeal fracture model and the results compared with two commonly used internal fixation devices (lateral plate, crossed K-wires) in a cadaver model. Each fixation technique was tested for its biomechanical strength in apex palmar bending, compression and torsion. Failure testing for the three resorbable methods was also done. The results showed that lateral plating provided the best rigidity in apex palmar bending and torsion, followed by intramedullary bone peg fixation. All resorbable intramedullary fixations had rigidity that was at least the same as crossed K-wires. For the torque test, polyglycolide rods with interlocking provided better rigidity than without interlocking. There was no significant difference between the different methods in the compression test, except that the intramedullary bone peg was significantly stiffer than K-wires.

A combined AFM and nanoindentation technique to investigate the elastic properties of bone lamellae

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Introduction: This study aims at extending the increasingly well understood relationships between structure and function of bone by investigating the mechanical properties of single lamellae. The results are expected to bring insight in bone lamellation theory, the mechanical environment of bone cells and contribute to a better understanding of metabolic diseases.
Materials and Methods: A preliminary cortical bone specimen was cut from a bovine femoral diaphysis, polished with silicon carbide paper and finished with 0.25mm diamond paste. A combined atomic force microscopy (AFM) and nanoindentation technique was utilized to measure Young's modulus and hardness. First, topography of a 45 x 45mm window selected within an osteon was scanned in AFM-mode. Second, using the same tip, indentations were performed within single lamellae at a loading rate of 0.15mN/s and a depth of 450nm. Young's modulus and hardness were calculated from the force-displacement curves using a standard protocol. Finally, the exact locations of the indents were checked with a second AFM scan.
Results: Within a same lamella, Young's modulus and hardness were approximately constant, with averages of 16.4±0.9GPa and 0.62±0.07GPa respectively. Across successive lamellae, mechanical properties decreased with distance from the Haversian channel: Young's modulus decreased from 25.5GPa down to 16.3GPa, while hardness decreased from 0.64 to 0.49GPa.
Discussion: As expected, the combination of AFM with nanoindentation provided higher spatial resolution than the previously used optical microscopy with nanoindentation.

Non-destructive characterisation of surface topography of implant materials

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The implant surface topography is an important factor for bone fixation as have been shown in several experimental studies in vivo. To be able to characterise numerically and visually different surface topographies, three major groups of equipment are commercial available: mechanical contact profilometers, optical instruments and scanning probe microscopes. For soft and easily damaged materials, non-contact optical methods are preferable. Furthermore, for screw-shaped implants with a narrow pitch-height, optical methods are so far the only choice if also the screw-flank shall be measured in a non-destructive manner. Optical methods that can provide with such measurements are confocal laser scanning profilometers, and interferometry instruments. Besides studies published by the present author, few studies are found in the literature where optical instruments have been used for 3D mapping of screw-shaped implants. Obvious reasons are that the number of such commercial available instruments is limited and that they still are expensive. However, optical instruments are very promising for surface topographical characterisation of different biomaterials. They need no sample preparation and are therefore non-destructive, they can provide with the same parameter set as the contact stylus instruments and the measuring time is considerably decreased compared with contact instruments. Another important issue is the evaluation of the measured data. If results performed at different laboratories should be possible to compare, it is a demand that the filtering process is described as well as a clear description of used parameters.

Characterising fretting particles by analysis of SEM images

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Detailed surface characteristics of the particles produced during fretting may well be significant in determining their biological effects. Apart from a broad size determination, very few attempts have been made at devising means of describing profile textures of particles. A new method is described for measuring the nature of particle surface texture, as detected on the particle profile. Careful processing and analysis of the digitised image enabled both the sizes of projections and their relative numbers to be determined. Such processing of images of a large number of particles generated a considerable amount of data. An artificial neural network was used to categorise the data and to compare the nature of fretting particles generated by titanium, titanium-molybdenum and stainless steel. Titanium produced the greatest diversity of textures and sizes, steel the least.

Cell reactions to nano- and microtopography

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The advent of precision methods of making microtopography and nanotopography in a variety of materials has allowed an unprecedented expansion in experiments to test the reaction of cells to topography. Early experiments showed that cells orient to grooves and fibres but more recently a whole variety of topographies have been made and tested and found to produce a variety of reactions in cells. Nearly all animal cells tested react in some way to topography though the most effective dimensions for this show considerable differences from cell type to cell type. Some cells react to dimensions as small as 10nm. The reactions include changes in adhesion, cell movement, cytoskeletal orientation, tyrosine kinase activation and changes in gene expression.

These reactions play an important role in development and in the reactions that lead to tissue building and perhaps stabilisation in adult life as well as in wound repair. Tissue and cell engineering are starting to use the repertoire of topographical reactions in designing prostheses for tissue repair.
Finally, I shall discuss recent work on the cellular mechanisms underlying the sensing of and reaction to topography.

Measurement and Scale-dependent Evaluation of Implant Surface Topographies in the Micron- and Submicron Range

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Surface topographies of implants are highly relevant for the behaviour of the biomaterial surface in contact with adjacent tissue. Increased surface roughness, achieved through processes such as particle blasting, plasma spraying or chemical/electrochemical etching are commercially used for titanium implants to promote bone integration and long-term stability of the implant in the patient. However, both the measurement and the quantitative evaluation of implant surface topographies pose specific problems that are relevant for the development of tailored surfaces as well as for quality assurance purposes. Both aspects are addressed in this contribution.

Blasted, etched and blasted + etched titanium implant surfaces were measured with non-contact laser profilometry (LPM), interference microscopy (IFM) atomic force microscopy (AFM) and stereo-SEM. These techniques differ highly in respect to lateral and vertical resolution, artefacts and measuring time in 2D and particularly 3D mode. Stereo-SEM is shown to be complementary to LPM, IM and AFM and of particular value for the characterisation of surfaces with ‘sharp’ surface topographies.
Conventional, 'integral' roughness parameter sets are of very limited value in describing the complex surface structures, since the surfaces often show a variety of topographical features across the nanometer and micrometer ranges, believed to be relevant to the interaction of the surfaces with biomolecules such as proteins, with cells and with tissue. Wavelength-dependent roughness is shown to be a successful method for the description of surface topographies in various characteristic roughness ranges, as well as being a useful indicator of the effect of surface treatment processes.

The surface topographical data in the various, predefined size ranges of biological relevance have been correlated with in vitro and/or in vivo biological performance data.

Mechanical properties of bone cells

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It is now accepted that cells are sensitive to deformations. The sensing system is only partly understood and the structure of the sensor itself and where it is located is unknown. However some properties are known. One property especially interesting for the biomaterials world is that very high deformations (over 10% of the cell length, or a movement of 2 micrometers) that perhaps occur at interfaces of two dissimilar materials, such as bone and a biomaterials, cause the cell to reprogramme its genetic machinery a re-differentiate into a completely different type of cell, producing matrix components consistent with the hypothesis that this mechanical re-programming induces directly the fibrous capsule. Other parameters, such as the mode of strain (compression, uniaxial, biaxial) the frequency and the biochemical pathways involved in mechanosensing will be presented in addition.

Influence of doping Sol-Gel Derived Titania coatings on proliferation and differentiation of bone marrow cells

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Sol-gel derived titania can be used to create coatings mimicking the oxide layer of titanium [1]. These coatings have proven to be highly biocompatible [2]. However, the mechanism of interaction between the titania surface and cells is not yet fully understood. To analyze the influence of dopant addition on the proliferation and differentiation of bone marrow cells, Al^{3+}, Zr^{4+}, Ta^{5+} were added as dopants during the preparation of the sol-gel derived titania coatings.

Coatings were applied on glass cover slips by dip coating. Protein adsorption was characterized by gel electrophoresis after incubating the samples for 12 h in fetal bovine serum (FBS, Gibco, Switzerland). The coated substrates were seeded with freshly isolated adult male rat bone marrow cells (500000 cells/ml) in medium (a-MEM medium, Gibco, Switzerland) supplemented with 10% FBS and 1 % antibiotics. Cell cultures were incubated at 37 _C, 5% CO_2, 95 % humidity. After a culture period of 14 days, cell function (neutral red uptake, MTT conversion, protein content) and cell differentiation (alkaline phosphatase, tartrate acid phosphatase activity) were measured.

[1] S. Kothari et al., Sol-Gel Titania Surfaces for Medical Implants, Part 2: In Vitro Evaluation, Ceramic Films and Coatings, W. E. Lee (ed.), the Institute of Materials, 1995
[2] D. Haddow et al., Synthetic implant surfaces, Biomaterials 17, 501-507, 1996

Osseointegration of copper vapour laser structured implants for hip endoprothesis – cell culture and animal experiment investigation

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In the study presented, copper vapour laser structured implants with pore sizes of 25 to 500µm were investigated in regard of effects on osseointegration, especially in the field of hip endprothetic surgery.
Material and method: In vitro biocompatibiliy tests were performed with an human osteoblatic cell line (hFob 1.19). Cell culture examination were performed using 50 cpTi disc with pore sizes of 25µm, 50µm, 100µm, 200µm, 300µm, 400µm, 500µm. The pores were visualised measured by backscattered electron microscopic examination. Test criteria in cell culture were the number of cells, cell vitality (WST-Test) and the alkaline phosphatase production.

The animal experiments were performed using 60 male, adult new Zealand white rabbits. Implants with pore sizes of 25 µm, 50µm, 200µm and a surface blasted industrial standard (Ra = 7,25µm) were selected for the animal experimental investigations. The implants (TiAl6V4) were placed randomly in both humeri and both femora of each rabbit in a diaphyseal position in the intramedullary canal. After a study time of 3, 6 and 12 weeks all the implants were analysed by histologic, histomorphometric, fluorescence microscopic, microradiographic and biomechanical methods.
Results: Cell culture results demonstrated that biocompatibility was given in all implants except the 100µm pores. The best results were obtained with the discs with 25 and 50µm pores. However, animal experiments showed advantages for the 200µm pores, especially in the fluorescence microscopic and biomechanical examinations.

Animal models of osteoporosis-necessity and limitations

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There is a great need to develop an animal model for postmenopausal osteoporosis; a model that will be useful for the understanding of the pathogenesis of the disease, investigation of new therapies (e.g. SERMs) and evaluation of prosthetic devices in osteoporotic bone. There are certain requirements for such an animal model and ones that have been used in the past include non-human primates, dogs, cats, rodents, rabbits, guinea pigs and minipigs. Sheep are a promising model for various reasons; they are docile, easy to handle and house, relatively inexpensive, available in large numbers, spontaneously ovulate and the bones are large enough to evaluate orthopaedic implants. Nevertheless, all animal models have advantages and disadvantages. More recently, interest in discovery of appropriate prosthetic devices which would stimulate osseointegration into osteoporotic, appendicular, axial and mandibular bone has intensified. Augmentation of osteopenic lumbar vertebrae with bioactive ceramics (vertebroplasty) is another area that will require testing in the appropriate animal model. Experimental animal models for the study of these different facets of osteoporosis eliminate the pitfalls associated with studying the disease in humans, namely time and behavioural variability among test subjects. The elimination of such variables would result from the use of carefully chosen animal groups. New experimental drug therapies and orthopaedic implants could be tested on large numbers of animals maintained with a level of experimental control impossible in human clinical research.

Implant surface modified by a laser technique. An *in vivo* study in rabbits.

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The aim of the present study was to produce a controlled surface topography with a laser technique to be able to investigate different aspects inherent in surface roughness (i.e height and space).
Materials and Methods: 60 c.p. titanium implants (Nobel Biocare, Sweden) with a length of 10 mm, outer diameter 3.75 mm and a pitch height of 0.6 mm were used. 30 were left with an as-machined (turned) surface and used as control implants. The remaining 30 implants were patterned on the screw flanks by a laser technique. The surface topography characterisation was made by use of an optical profilometer. The implants were inserted in the femur and the tibia of 10 rabbits. Healing period was 12 weeks.
Results: The numerical characterisation demonstrated 2 different surface topographies:

	Sa (µm)	Scx (µm)	Sdr
Patterned	1.29	11.71	1.41
Control	0.65	8.01	1.22

Sa: Mean arithmetic height deviation from the mean plane.
Scx: Mean space between the individual irregularities crossing the mean plane.
Sdr: Ratio of developed area.

The evaluation from the removal torque and the results from the histomorphometrical evaluation analysis showed no significant differences between the test and control implants. This may to part be related to problems to achieve a well focused pattern on a screw-shaped design. The laser technique needs to be improved in further investigations.

Characterization of pure titanium surfaces after different types of oxidation

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Surface properties are crucial for interactions at the tissue interface. Specimens representative of titanium implants have been produced to analyze physico-chemical surface properties of differently machined and oxidized pure titanium surfaces. This permitted the determination of surface-treatment attributes as well as oxidation variations like spontaneous oxidation, anodic oxidation (30, 57, 90V) after pickling or thermal oxidation (280, 400^{o}C for 3 hours) following passivation and considering sterilization.

Contrary to SEM, the surface roughnesses determined by profilometry did not provide very big differences. According to TEM with increasing extent of oxidation, the at first amorphous layers revealed more and more crystalline portions of the

lattice structure anatase, and also partly rutile in thermally oxidized specimens. XPS combined with sputtering by Ar^+ ions showed that the surface treatment can significantly influence the chemical properties of the surface layers characterized as TiO_2. The principal top layer components were Ti (9-27at%), O (35-59at%) and C (12-45at%). Residues (Fe, Al, Cr, Ni) from specific surface treatments could be found in the thinner oxide layers. P (2-13at%) was detected in the top layers as a result of the anodic oxidation. The thickness of oxide layers was increasing from spontaneously oxidized specimens (10-20nm) to thermally oxidized ones (30-70nm) to anodically oxidized ones (90-300nm).

The choice of individual manufacturing steps allows to influence the chemical composition, the thickness and the lattice structure of pure titanium surface layers but also the surface roughness. It should be possible to further optimize these variations with regard to a physiological integration of implants.

Characterisation of small particles of hydroxyapatite by high resolution Transmission Electron Microscopy and diffraction.

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At the present time the increasing demand for synthetic bone replacement materials and feed additives requires the accurate characterisation on the microscopic level of hydroxyapatite (HAP), one of the most important bioimplants.

The phase composition, the particle morphology and size of calcium phosphate precipitates obtained from aqueous solutions are directly correlated with their growth conditions: temperature, pH, concentrations, local supersaturation and rate of mixing of the initial reagents. Moreover, both mixtures of several calcium phosphates and mixed multiphase crystals can be formed instead of a pure substance. Therefore, the knowledge alone of the calcium to phosphorus ratio (Ca/P) in a precipitate is not sufficient to assess phase composition. For instance a Ca/P ratio less than 1.67 does not indicate the absence of HAP, but result most often from a mixture of brushite and HAP or octacalcium phosphate (OCP) and HAP. In this latter case the close similarity between hexagonal HAP and triclinic OCP makes quite difficult to distinguish one structure from another using X-ray diffraction only.

Specimens of HAP produced under various conditions have been examined by HRTEM and electron diffraction. It was found that all the HAP particles were thin platelets elongated in the **c** direction with different sizes ranging from a few nanometers to a few micrometers. Nanometer-sized electron probes provided the mean to establish that even the smallest HAP particles, which were in solution not more than 1 min, behave a clear crystalline character, although they were considered as "amorphous" from conventional SAED and X-ray diffraction. HRTEM lattice images also exhibit the fine structure of the HAP crystals and leads to an estimation of their thickness.

Dental Biomaterials of the Future

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Dental biomaterials range from alloys to ceramics to resins and composite materials, with applications as diverse as replacement of part of a tooth (fillings), or of tooth or teeth (crowns and bridges, prostheses), as well as clinical and technical means to achieve the said replacements (impression materials, luting cements, gypsum products, etc.), and as preventive measures (pit and fissure sealants). These very specific biomaterials have called for specific design systems or in situ transformation processes, since most of them are used directly (chairside).

Recent trends in public awareness, like demand for improved aesthetics, and increased importance of the biocompatibility through the new European directives on medical devices, have initiated the development of new families of biomaterials and fabrication technologies for use in dentistry. As an illustration of these tendencies, two topics will be presented: the replacement of dental amalgams for fillings, today and in the near future, and advanced designs and technologies for the fabrication of bridges, with and without a metal framework.

Metallic and allegedly toxic amalgam fillings can now be replaced by composite resins or by ceramic inlays. The latest developments in that area, even those still experimental, will be reviewed, including innovations in the chemistry of resins, and new methods of inlay fabrication, either chairside or at the dental laboratory. New materials and CAD/CAM systems for the making of crowns and bridges will also be presented.

Resolving nanometric fluctuations of biopolymers with digital video light microscopy: An efficient way to study architectural dynamics of living cells.

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In the shadow of Electron Microscopy and near-field sensing, conventional light microscopy (LM) has experienced revolutionary advancements. Compared to these other sensors, the LM bears two advantages: 1.) It is largely non-invasive and 2.) it provides relational data over large areas in real-time. Both capabilities make the LM attractive for studies of dynamic processes, e.g. in biological tissues.

Three factors have contributed to the recent improvement of LM: Novel contrast modes, electronic imaging, and digital image processing. Altogether, they have boosted what is commonly considered the resolution limit of LM and have made the seemingly invisible visible. This talk will report two examples where contrast modes in conjunction with electronic imaging and image analysis have unveiled biological mechanisms at the macro-molecular level. The key to both results was the efficient combination of high NA LM with computer vision such that nanometric fluctuations of linear, molecular structures became quantifiable.

In the first application we have measured Brownian motion of micro-tubules imaged with video enhanced differential interference contrast (VE-DIC) microscopy. The measurements were used to derive the rigidity of these biopolymers, which is a fundamental parameter for an important building block of cell architecture.

In the second application we visualized actin traffic in the lamellipodium of motile cells using a new type of polarized LM (Pol-Scope). Actin plays a critical role in many molecular mechanisms underlying cell motility. What we see in the Pol-Scope is actually neither the actin monomer nor the single filament but the intrinsic birefringence of protein bundles of 10 or more filaments. By analyzing
the minute orientation fluctuations of these bundles we have devised a method for quantifying the flow field of actin filaments in the entire growth cone of an advancing nerve cell.

Chemical Analysis of Engineered Biosurfaces

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The interaction of cells with solids can be controlled by defined chemical properties of the top surface layers. This presentation introduces surface analysis methods like X-ray Photoelectron Spectroscopy (XPS) and Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) and how such advanced tools with high surface sensitivity (below monolayer coverage) and (sub-)micron lateral resolution allow us to control engineered biosurface modification. Presented applications will cover soft and hard biomaterials such as functionalized biomimicking polymers and controlled surface glycoengineering on inert surfaces, relevant in biosensing and biomedical applications.

Evolution of Microstructural Morphology in Ultra High Molecular Weight Polyethylene

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As morphological characteristics determine the physical properties of a material, we traced the evolution of the crystalline morphology of UHMWPE with the intent to understand more about the microstructure of damage and wear mechanisms in this material. Both virgin and damaged UHMWPE (machined GUR 415, gamma irradiated in air) have been analysed using transmission electron microscopy (TEM). Specimens with mechanically cracked surfaces as well as debris retrieved from failed total knee replacements (Zimmer® ,Miller-Galante knee prosthesis) have been examined. Microstructural architecture, lamellae thickness, lamellae orientation, and tortuosity were evaluated in each sample. Changes in UHMWPE morphology demonstrated considerable unidirectional re-orientation of the lamellae as a result of cracking. The irregular structure of the lamellae in the damaged specimens may reflect a lamellae defect. We found in this study that artificially damaged (cracked) samples showed different morphology of the crystalline regions than those evaluated in the in vivo delaminated tibial inserts. These less obvious changes in the retrieved inserts may demonstrate the difference in microstructural failure mechanism. Our data support the hypotheses that microstructural changes in morphology have important implications for the fatigue behavior of orthopaedic grade UHMWPE. Although the damage and failure modes in retrieved UHMWPE will require further research to elucidate completely, investigations like that could lead to a better understanding of microstructural features of retrieved UHMWPE and help to improve the performance as a weight-bearing material.

Chemical and Morphological Changes of Surface Treated Titanium Alloy Implants during Immersion in Bovine Serum Solution

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Ti-6Al-4V alloy has proven to be a popular biomaterial due to its good mechanical properties, excellent corrosion resistance and low toxicity. The excellent corrosion resistance is due to the titanium oxide surface film, which forms spontaneously on exposure to air. However, when the alloy is implanted into a complicated and aggressive physiological environment, its stability may be affected, resulting in increased metal ion release. The present research aims to improve the biocompatibility of the native oxide layer by applying simple surface treatments, such as nitric acid passivation treatment and ageing in boiling water. XPS has demonstrated the elimination of vanadium from the oxide layer after both treatments. AFM has demonstrated a noticeably rougher surface morphology for the aged samples immediately after treatment. This is thought to be due to the increased concentration of hydroxylated groups. The morphology of the immersed surfaces varied with immersion time for both treatments as large-scale agglomerate precipitates appeared on the surface. After 240 hours immersion, the surface roughness gradually decreased as the proteins spread across the metal surface. XPS analysis has shown that the magnitude of the TiO2 peak increased up to 10 hours immersion for both treatments. However, the peak continued to increase until 240 hours for the passivated samples only, as further oxidation and dissolution processes occurred. This explained the improved protein adsorption demonstrated by titanium implants, which had formed a stable, biocompatible oxide as a result of the simple ageing treatment.

Porous Polyesterurethane Foams

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In the present investigation, primary rat osteoblasts and the osteoblast cell line MC3T3-E1 were used to examine the possible use of DegraPol®/btc, a novel biodegradable and highly porous open-cell polyesterurethane-foam (pore size 100-300 µm), as bone repair materials.

Primary rat osteoblasts and the osteoblast cell line exhibited normal cell morphology and relatively high attachment and a doubling time of about 5 d. Eight days after cell seeding, osteoblasts exhibited a confluent cell multilayer and migrated into the pores of the polymer. In addition, they produced high concentrations of collagen type I and expressed increasing alkaline phosphatase activity and osteocalcin production throughout the 12 days of the experiment. Treatment with Vit. D3 led to a dose- and time-dependent increase in the ALP activity and osteocalcin concentration. Compared to control cells, ALP and osteocalcin were increased to about 1.5-2 fold and 0.3-0.5 fold, respectively. In contrast, Vit. D3 treatment led to a decrease in the cell density and collagen type I concentrations. Compared to untreated osteoblasts, Vit. D3 decreased the cell density and collagen type I concentrations by about 5-35% and 10%-40%, respectively.

Results obtained in the present study indicate that Degrapol®/btc foams exhibit good osteoblast compatibility; osteoblasts showed relatively high cell adhesion, growth rates, maintained their phenotype for up to 12 days, and responded to 1,25-dihydroxyvitamin D3 treatment. This points to the possible use of this scaffold in the bone healing process.

Interaction of chondrocytes with DEGRAPOL® structures, biodegradable and highly porous polyesterurethane foams

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Cartilage defects occur in a wide variety of clinical situations. An ideal surgical reconstruction repairs the defect with autologous tissue. However, because of the limited quantity of usable such tissue as well as undesirable donor site morbidity, engineering of biomaterials that enhance formation of neo-cartilage is desirable. In the present in vitro investigation primary bovine- and rat- chondrocytes were used to examine the possible use of DegraPol/btc®, a novel biodegradable and highly porous polyesterurethane-foam (pore size 100-300 µm), as substrate for the formation of neo-cartilage.

Primary isolated bovine costal- and rat xyphoid-chondrocytes attached to the polymer scaffolds exhibited a rounded morphology when examined by phase contrast microscopy. Six days after cell seeding, chondrocytes formed a confluent cell multilayer when examined by SEM. Further histologic sections showed large cell ingrowth into the pores of the foam. Additionally, aldehyde fuchsin pH 1.7-alcian blue stains revealed a matrix that stained deeply purple consistent with the presence of chondroitin sulfate. Chondrocytes cultured on the foam produced relatively high amounts of collagen type II and chondroitin sulfate.

Results obtained in the present *in vitro* study showed that chondrocytes cultured on DegraPol®/btc foam exhibit relatively high cell attachment and maintained their phenotype for up to 12 days, This points to the possible use of this scaffold as substrate for the production of neo-cartilage.

Atomic force microscopy (AFM) as a tool for quality control of engineered cartilage. A feasibility study.

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Engineered cartilage may replace the damaged tissue in patients with impaired cartilage function due to acute (e. g. trauma) or chronic (e.g. osteoarthritis) tissue damage. Efforts to grow cartilage in vitro have been successful, but have yet to yield tissue with biomechanical properties equivalent to native tissue. The mechanical properties of cartilage relate to the composition and the spatial arrangement of the extracellular matrix (ECM), i. e. mainly collagens and proteoglycans.

Comparative analysis of native and engineered tissue, a prerequisite for documenting bioequivalence, is commonly performed by light and electron microscopy and by biochemical analysis, and functional assessment by mechanical measurements. However, conventional microscopy techniques include preparation steps that can alter the tissue fine-structure and the collagen fibre diameter in particular. In contrast, atomic force microscopy (AFM) can be performed in water and at physiological conditions. Moreover, AFM measurements also relate to local mechanical properties. Here, we show that AFM can be adapted to analysing different types of cartilage (porcine: ear, knee, vertebral disc) with respect to ECM organisation and elasticity. Image resolution was at least comparable to that obtained by SEM and overall elasticity was in accordance with reported values based on compressibility measurements.

Direct visualization of the soft tissue-implant interface by virtual sectioning. An in vitro study using Reflection/Fluorescence-Confocal Laser Scanning Microscopy (R/FL-CLSM) and Scanning Electron Microscopy (SEM).

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There is growing evidence that the surface texture of a surgical implant influences cell differentiation in the adjacent tissue. Yet, the visualisation of tissue interacting with metal surfaces is limited by the very divergent nature of the components. Traditionally, tissue/ implant interfaces are inspected indirectly by means of cross sections using light microscopy (LM) or transmission electron microscopy (TEM). Alternatively, scanning electron microscopy (SEM), or CLSM operating in the reflection mode (R-mode) provide a three dimensional view of the surface texture, while CLSM in the fluorescence mode (FL-mode) reveals the spatial arrangement of specifically labelled tissue constituents.

Here, we combined the R-mode and the FL-mode to directly document the influence of the surface texture on cell differentiation. We emulated the interaction of endostial implants with the surrounding tissue by growing periodontal ligament cells (PDL) on titanium disks having a polished (MP), a Ti-plasma sprayed (TPS) or a hydroxyapatite-coated (HA) surface. At a specific setting, high resolution SEM revealed either the cell surface or the underlying metal texture. In contrast, R/FL-CLSM, i. e. concomitant registration in the R-mode and in the FL-mode, enabled us to directly visualise the spatial architecture off cells and fluorescent phenotypic markers such as cytoskeletal elements and extracellular matrix proteins in relation to the underlying surface.

A New Polyurethane-Based Adhesive for Injured Bones: I. An In Vitro Characterization Study

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The new composite is a reaction product of a commercial di-isocyanate with aliphatic oligoesters that were synthesised in our laboratory. The final polymerisation product (following 24 h in saline at room temperature) has a 3-D porous configuration with a total pore area of about 25% and an average pore size of 100-200 mm in diameter. The temperatures generated during the polymerisation process differed in the various formulae used. By 10 min. temperatures stabilised at 31°-39°C. The adhesives' strength was 0.8-0.9 MPa. The new adhesive was found to be highly sensitive to the proteolytic effect of the enzyme papain. This enzyme at a concentration of 50 U/ml led to a 30-50% decrease in the bonding strength of the adhesive. Chymotrypsin at a concentration of 100 U/ml also brought about a similar magnitude of weight loss that started only following 60 days of incubation. The present study checked diamine groups that were released from the polymer into the incubation media in vitro, using UV spectroscopy. Both papain and chymotrypsin induced a degradative effect immediately following the onset of the incubation; an effect that lasted until the termination of the experiment by 200 days. Storing the adhesive's components at -18°C for a period of up to 2.5 months did not affect their polymerisation kinetics nor the strength of their polymerised compound.

A New Polyurethane-Based Adhesive for Injured Bones: II. An In Vivo Biocompatibility Study

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Young female beagle dogs served as the experimental model. Osteotomies were performed in the frontal and parietal bones, and the bone fragments were completely detached from the cranium. The adhesive has been applied as a thin layer over the exposed bone surfaces and the bone grafts were then installed back into their original sites. In situ, the compound polymerised for 10-15 min prior to obtaining sufficient stability in order to allow the closure of the operative sites. Animals were followed to up to 24 months postoperatively via blood tests, x-rays (CT), bone biopsies and histological examinations of selected internal organs. Structural analyses of bone biopsies revealed clear signs of biodegradability concurrent with de novo fibro- and osteogenesis. By the end of the experimental period the operated sites still contained remnants of the adhesive yet the osteotomized bones re-united through well developed new bony bridges. Successive haematology tests indicated a transient inflammatory reaction which subsided within weeks. Tissue biopsies from liver, kidney, lung, spleen, lymph nodes and others were free of pathological manifestations. The newly devised compound proved, in a long-term in vivo study, to serve as an efficient bone adhesive material that is being gradually degraded by its host while enabling new bone formation in its vicinity. Both locally and systematically the compound lacked any adverse reaction, thus appeared compatible.

Ultrastructural and compositional characterisation of the dentin-cementum interface: natures approach to resist high tensional forces.

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The presence of a distinctive matrix layer at bone-bone (cement line) and bone-implant interfaces implies that the structural and compositional constitution of such an interfacial layer has a functional significance. Common to these two interfacial layers is that they lack collagen fibrils and contain noncollagenous matrix proteins (NCPs), such as bone sialoprotein (BSP) and osteopontin (OPN), that may mediate the local cell dynamics, and contribute to regulating mineralisation and imparting tissue cohesion. It has also been suggested that these matrix layers may provide a ductile interface that dissipates the effects of loading.

The absence of a cement line-like structure between dentin and cementum in human teeth, however, suggests that there are also other binding mechanisms between mineralised tissues. Ultrastructural and immunocytochemical analyses in human teeth show that a collagen fibril interdigitation between predentin and precementum precedes the deposition of BSP, OPN and mineral. As a consequence, BSP and OPN fill up the interfibrillar spaces. These observations in human teeth indicate that, although cement lines are normal features in bone, they are not a prerequisite for tissue integrity in all calcified tissues. This may be due to a different strain distribution in bone and teeth. The dentino-cemental junction has to withstand high tensile strengths generated during mastication, speech and parafunctional stress (tooth pressing and grinding). While the dentino-cemental junction itself appears to represent a rigid interface, the periodontal ligament, the soft connective tissue that spans between the root and the surrounding alveolar bone may execute the function of a physical buffer.

Osteoblast attachment and growth on microfabricated model surfaces with chemistries relevant to titanium alloy orthopaedic implants.

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The clinical success of any implant is directly dependent upon the cellular behaviour in the immediate vicinity of the interface established between the host tissue and the biomaterial used to fabricate the device. All biomaterials have morphologic, chemical, and electrical surface characteristics that influence the cellular response to the implant. Microfabrication has proved to be a very valuable tool for producing geometric patterns with well-defined surface chemistry and topography.

In this study, model surfaces with spatially patterned regions of Ti, V, Al and Nb produced by microfabrication technique were used to study the chemical influence on human osteoblast attachment. The oxides to be studied were selected according to their relevance for the application of titanium and titanium alloys as medical implants: TiO_2, Al_2O_3, V_2O_5, Nb_2O_5; these oxides are known to be present at the surface of titanium implants and to form a passive layer protecting the surface from corrosion.

Primary human osteoblasts, derived from femoral head trabecular bone were seeded onto surfaces at a density of $3x10^4 cm^{-2}$. These were maintained in DMEM with FCS. Cells were fixed at appropriate time points and permeabilised prior to incubation with a 1° antibody. 1° antibodies used were against β tubulin, vinculin and focal adhesion kinase.

Variations in the chemistry of the model surfaces used in this study clearly influence cell behaviour. Vanadium and aluminium exert the greatest influence on cells, influencing cell shape and cytoskeletal organisation and supporting inferior cell growth relative to the other component of the pattern respectively.

XPS analysis of coatings and materials for biomedical applications

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The chemical surface composition of an implant is of prime importance for the reaction of the tissue surrounding the implant (e.g. small amounts of contaminants can render an implant unusable).
X-ray photoelectron spectroscopy (XPS) is shown to be a very valuable surface analytical tool to investigate the outermost surface of implant materials and modified biomaterials. By analysing the surface chemistry of plasma treated or coated biomaterials the information can be further used for tailoring biomaterials.

Polyethyleneterephtalate (PET) meshes were plasma treated in an oxygen plasma for different durations. The meshs, used for cultivating adult rat ventricular cardiomocytes, were dipped in a 0.1% solution of Gelatin from porcine skin in water. XPS revealed that prolonging the duration of the plasma activation is directly proportional to the amount of nitrogen found on the plasma activated and gelatine exposed meshes and the observed increasing cell attachment.

XPS analysis of a-C:H/Ti coatings with different Ti concentrations reflected the formation of TiO_2 and TiC of the coating. Protein adsorption measurements of those different films showed a correlation between the adsorption of certain proteins to the amount of $Ti(O_2)$ incorporated on the coatings surface.

The amount of Ti incorporated into the a-C:H matrix also had a positive influence in the formation of osteoblasts.

Similar results were found for a-C:H/V coatings, i.e. the formation of an oxide on the surface and a carbide in the films. Possible applications of these cell toxic coatings are the coating of short term implants or sensors where a biofilm formation must be prevented.

Histotomography of Fresh Samples of Human Tissue by Confocal Laser Scanning Microscopy

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Introduction: In standard microscopy the study of hard tissues requires sample preparation, e.g. grinding, decalcification, etc. with an inherent risk of artefacts. In consequence, microscopy of fresh untreated samples of hard tissues would be preferable. Method: Confocal laser scanning microsocopy in reflection mode (Leica-CLSM Aristoplan) equipped with an Ar/Kr Laser, 488nm. Samples: a) Sound human hard tissue (cortical jaw bone n = 20, teeth n = 50. b) Pathological bone and teeth from patients with head and neck cancer affected by radiotherapy (cortical jaw bone n = 40, teeth n = 250). Subsurface areas (? 20 µm under the surface) of specimens were studied as fresh blocks and after embedding in MMA-resin. As a control identical areas of the above samples were visualised by conventional light microscopy.

Results: In reflection mode, CLSM allows to identify osteocytes and lamellae in untreated fresh and embedded bone samples. The structures correlate with those from conventional light microscopy. Cytoplasmatic processes of osteocytes are visualised in high contrast, whereas subcellular structures seem to be problematic. In tooth specimens the prismatic structure of enamel, detinal tubuli as well odontoblast processes can be visualised with high contrast. After radiation with 36 Gy a loss of vitality of the osteocytes and irregular structure of the cortical lamellae are typical. Radiated teeth showed a retraction of the odontoblast processes.
Conclusion: CLSM in reflection mode proved to be an alternate technique for non-destructive histology of hard tissue. The risk of artefacts due to pre-treatment is minimised by the option of sub-surface microscopy of fresh untreated hard tissues.

DEXA, QCT and Histomorphometry in the evaluation of fracture risk in osteoporotic women. A comparative analysis.

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Introduction: In this study qCT and DEXA were compared to histomorphometric analysis regarding their accuracy, sensitivity and specificity in the assessment of bone mineral density in osteoporosis
Material and methods: From 20 female patients, who had experienced a medial femoral neck fracture BMD of the removed femoral head after THR was measured using DEXA and classical histomorphometry was performed. The patients underwent DEXA of the contralateral proximal femur and qCT of the second vertebral body (lumbar spine).
As a control, the femoral head, the second vertebral body of 20 female patients were harvested following autopsy and the same measurements were performed as described above.
Results: DEXA and qCT showed almost identical results when BMD was measured at the proximal femur site. Histomorphometry results could not be correlated to those in both groups. At the lumbar spine again DEXA and qCT measurements were closely correlated. In contrast to the measurements at the femur, histomorphometric analysis in the cadaver study showed a high correlation to BMD values obtained by either technique.
Conclusions: QCT and DEXA measurements of BMD are reproducible and data of both methods can be readily compared. Although, measurements taken at the proximal femur show no correlation to classical histomorphometrical analysis and BMD data, when obtained from these sites seems questionable in the light of our results.

Both techniques are much more powerful as a tool for the assessment of osteoporosis at the lumbar vertebral body, where they show a much better correlation to histomorphometric data.

Direct visualisation of immunogold-labelled vinculin within focal adhesion sites on the undersurface of fibroblastic cells .

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An understanding of cell-substratum adhesion to biomaterial surfaces is of value in promoting the attachment of connective tissue onto implant materials. In addition, adhesion of cells to implant surfaces is one of the major factors which determines the implant's biocompatibility. The current definition of biocompatibility is that it refers to the ability of the material in question to perform with an appropriate host response in a specific situation (Williams, 1987). In certain circumstances on implant surfaces it is important to have adhesion of the soft tissues to prevent capsule formation and in other circumstances it may be desirable to have no adhesion, such as where tendons cross implants. Therefore measuring adhesion of appropriate cells and tissues to implant surfaces is very important for helping to screen new implant surface designs.

Previously a semi-quantitative method of measuring the amount of cell adhesion was shown. This was based on general heavy metal staining, scanning electron microscopy (SEM) and image analysis of both the whole cell shape and the stained areas at the cell-substrate interface(Richards *et al*., 1996). Also Hunter *et al*., 1995, studied cell attachment using indirect immunofluorescent labelling of vinculin, quantifying the degree of cell attachment by determining the mean number of focal adhesions, containing vinculin, and determining the mean total area of the focal adhesions per cell.

We now report an improved method for indirect immunocytochemical labelling of the integral protein vinculin within the cell focal adhesions for SEM viewing. The focal adhesions are either viewed, with the SEM, from above with the cells still on the substrate or from below, after the cells have been embedded in resin and the substrate removed.

The advantages of this method over immunofluorescent labelling are: the possibility of high resolution viewing, there is no possibility of colour fading or autofluorescence problems - samples can be kept for years since gold particles do not disappear and also because of the particulate nature quantitation can be more accurate.

There are two main advantages of this new method over the standard indirect immunolabelling for SEM viewing.
1. The literature (Beesley, 1993) suggests blocking (with the non-immune serum from the species that provides the

secondary antibody) to be performed before applying the primary antibody. We show that non-specific labelling is lower if this is performed just before the secondary antibody is applied.
2. Detergent extraction (using Triton X-100) is performed before the fixation. This is possible since it is known that Triton X-100 doesn't extract vinculin from focal adhesions (Niederreiter et al., 1994). By this step most of the cell upper layers are removed allowing an easy visualisation of adhesion patterns in SEM with very low background labelling.

The method will now be developed with image analysis techniques to allow a more accurate quantitative comparison of cell adhesion to different materials.

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Carbon materials in the treatment of hard and soft tissue injuries – Revisited

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Carbon – based implant materials are of interest due to their acceptance by biological environment. Good biocompatibity of particular form of carbon implants, namely carbon fibres and carbon fibrous composites with osseous and soft tissues has been confirmed in numerous studies.

Carbon fibrous materials developed in the Department of Advanced Ceramics (UMM, Cracow) were tested in vitro and in vivo studies aimed at the determination of their influence on the living body.
This work is devoted to the synthesis – structure – properties relationships of fibrous carbon implants with respect to the tissue response. It is shown that tissue response depends on material form, crystallite ordering degree, concentration of heteroatoms and microstructure parameters. Qualitative correlation between chemical factors of carbon structure and the tissue response to the implant is determined. Examples of clinical applications of carbon implants in orthopaedics, surgery, dermatology including soft and hard tissue repairing and reconstruction are shown.

Submicrometric organization of collagen on polymer surfaces and influence on the attachment of mammalian cells

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Combination of atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS) and adsorption measurements by radiolabeling has shown the influence of substratum surface properties on adsorption of extracellular matrix proteins in presence of competing compounds. This allowed controlling the selective adhesion of mammalian cells on oxidized tracks on polystyrene. The same methodology has shown the influence of substratum roughness and hydrophobicity on the organisation of collagen layers adsorbed in absence of competitor.

The influence of drying on the organisation of collagen adsorbed on poly(methyl methacrylate) (PMMA) was also investigated. While a continuous layer of collagen was observed at fast drying rate, a net-like structure appeared at slow drying rate. Further investigation using XPS and water contact angle measurement allowed concluding that PMMA is exposed at the outermost surface in the holes of the collagen net. Chemically heterogeneous surfaces presenting an alternance of collagen and polymer domains on the submicrometer scale are thus easily created. The stability in aqueous medium of the structures obtained by fast and slow drying was checked using AFM and dynamic contact angle analysis.

The influence of submicrometric-scale heterogeneity of collagen-conditioned PMMA on the behaviour of mammalian cells was then explored. The attachment of human tumoral cells was much better on heterogeneous collagen/PMMA surfaces than on homogeneous collagen layers. This shows that the organisation of adsorbed collagen layers on the subcellular scale has a direct influence on the adhesion of mammalian cells on solid surfaces; controlling this organisation opens new perspectives for controlling the behaviour of mammalian cells. The mechanisms leading to these different cellular responses are now under investigation.

Different osteoporosis induction modalities in a sheep model for fracture treatment in osteoporotic bone

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Sufficient fracture treatment in osteoporotic bone with conventional procedures is not always possible due to failure of fixation. There is a great need of a large animal model which allows the investigation of fracture healing and fracture treatment in mechanically weak bone. The aim of this pilot study was to compare different treatment regimes to induce osteoporosis in sheep during 6 months.

Methods

Eight Swiss mountain sheep, 7-9 years old, were divided into 4 treatment groups of 2 animals each.
Group 1: Ovariectomy (OVX) and calcium/vitamin D-restricted diet (O+D)
Group 2: Ovariectomy and 25 mg methylprednisolone i.m./day (O+S)
Group 3: Ovariectomy, 25 mg methylprednisolone i.m./day and calcium/vitamin D-restricted diet (O+D+S)
Group 4: Control, no treatment

Preoperatively and every 2 months the bone mineral density (BMD) was determined by quantitative computed tomography (QCT) at the distal radius. Biopsies were taken from vertebral bodies and femoral heads and the bone structure was determined by micro-CT (Tb.N, Tb.Th, Tb.Sp, BV/TV, DA). In vitro compression testing of the lumbar vertebrae was performed.

The control group showed no changes in BMD over 6 months. The greatest decrease in BMD was seen in group 3 (O+D+S) with a decline of 58% in cancellous bone and 22% in cortical bone. In the biopsies a prominent change of the values for the structural parameters was observed compared to the control group (Tb.N -53%, Tb.Th -63%, Tb.Sp +150%). In the compression test the biopsies of group 3 (O+D+S) had values of 40% less in stiffness compared to the control and 70% less in failure load.

The induction of severe osteoporosis in sheep is possible. There is relation between BMD, structural and mechanical properties. The most effective method to induce osteoporosis in sheep is the combination of OVX, calcium/vitamin D restricted diet and steroids.

Effect of Biomaterial Surface Properties on Bone Cell Behaviour

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The integration of structured implant surfaces into the surrounding tissue is of key importance for the applicability of biomaterials in tissue substitution. Bone tissue formation depends on the generation of adequate cellular signals that shift the balance between bone formation and resorption towards bone formation. Divergating results have been reported regarding the effects of microstructuring for example by sandblasting and electrochemical oxidation procedures.

In the present study we investigated the effects of microstructuring on the proliferation (DNA) and differentiation (ALP and TRAP activity, total protein) of adult rat bone marrow cells in vitro. For these studies polystyrene cell culture dishes with 10 different microstructured surfaces were prepared by injection moulding. The microstructures tested were in the µm range. Bone marrow cells of adult animals were used in order to be close to implant situation. Our results indicate that cell performance is strongly affected by surface modification.

Influence of cell isolation, cell culture density, and cell nutrition on differentiation of rat calvaria osteoblasts *in vitro*

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The immediate microenvironment of osteoblasts has a fundamental influence on the differentiation of these cells *in vitro*. It is therefore important to optimise culture conditions in order to recreate an *in vitro* environment as close as possible to the *in vivo* one. In the present study, we studied the effect of various cell isolation procedures, the influence of different cell culture density conditions, and distinct growing media on the *in vitro* differentiation process of neonatal rat calvaria osteoblasts.

Method: Cells were enzymatically isolated from bone pieces, or allowed to migrate out from explant cultures of periosteum-free calvariae. Cells were inoculated as a monolayer or micro-masses at $2x10^5$ cells/$10cm^2$ in serum containing BGJb, or DMEM with pyruvate (D+), or DMEM without pyruvate (D-). At 3 weeks cells were characterised ultrastructurally, histochemically by alkaline phosphatase (ALP) staining and van Kossa staining for mineral deposition, as well as biochemically by collagen extraction and SDS-PAGE analysis.

Results: After 3 weeks of culture as monolayer, only cells grown in BGJb formed dense ALP positive nodules and mineral deposition was observed. In contrast, cells grown as micromasses formed a multilayer extracellular matrix, which was ALP positive and showed mineral deposition independently of the growth medium used. The ultrastructure of cells under all conditions showed typical features of osteoblasts cultured *in vitro*, and abundant collagen fibril production, but mineralisation was only observed in cell monolayer cultures grown in BGJb and in micromass cultures. The main collagen synthesised by the different culture conditions was collagen type I, although some collagen type V was also observed.

Conclusion: Monolayer cultures cells should be kept in BGJb in order to obtain mineralisation without the supplementation of inorganic or organic phosphates which can induce artefacts. In contrast, the cells kept in micromass cultures are less sensitive to nutritional effects and mineralise in various growth media.

Polymeric Materials Compatibility with Low Temperature Hydrogen Peroxide Gas Plasma Sterilisation

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The use of combined hydrogen peroxide vapour and low temperature gas plasma for inactivation of microorganisms and the removal of gaseous hydrogen peroxide residue makes it the sterilisation method of choice for a wide variety of medical device categories. A relatively new technology, hydrogen peroxide gas plasma sterilisation has been accepted in the medical arena as an expedient and material friendly option to replace traditional decontamination/sterilisation methods for medical devices. Hydrogen peroxide gas plasma has been shown to be especially beneficial for the sterilisation of temperature sensitive polymeric materials. This technology has been enhanced and introduced into the industrial marketplace for terminal sterilisation applications of single-use medical devices, implantable devices and many biomaterials.

The compatibility of device component materials with the sterilisation process is critical to effective sterilisation. Testing of medical device component has been done to give guidelines of materials compatibility to device manufacturers interested in hydrogen peroxide gas plasma sterilisation technology.

Materials testing and examples will be presented in this paper, as well a description of the low temperature hydrogen peroxide gas plasma sterilisation technology.

Electron microscope observations on keratinocytes and fibroblasts cultured on novel collagen scaffolds.

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We have developed two classes of collagen scaffolds for clinical application in burns, and wounds of other pathologies. One type of matrix, is developed from the devitalized tissue (DT) of bovine origin, and the other one from the reconstituted collagen matrix (RCM) from bovine type I collagen. Cell cultures of human keratinocytes and fibroblasts have shown enhanced proliferation when grown on these substrata. In many instances, it has been observed that the geometry and / or architecture of a material influences the biological response evoked by it in vivo. Our objective was to test whether a differential response was evoked by the two classes of collagen scaffolds, to *in vitro* cell cultures of keratinocytes and fibroblasts. The changes in the diameter of the collagen scaffolds and data on Scanning Electron Microscopic observations on cell morphology are discussed. Considering these results a comparative evaluation of the two types of scaffolds for medical applications will be presented.

Plasma treatment induced topography on Ti and Ti6Al4V characterized by SPM

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We used Scanning Probe Microscopy with the objective to characterise and quantify the roughness changes on originally smooth and rough surfaces of pure and alloyed Titanium samples induced by a glow-discharge plasma treatment. Plasma treatment is used in a large variety of applications in the industry and in research. During the plasma treatment ions, radicals, and electrons are created in a gas of choice and these are bombarding and reacting with the sample surface immersed into the plasma. By using an inert gas and biasing the sample high energy ions can be used for surface cleaning and/or structural modification. Mechanically and electropolished commercially pure Titanium plates and mechanically polished Ti6Al4V alloy plates were used and subjected to an Argon plasma. Characterisation was done with an air operated AFM in contact mode and a UHV-STM. On electropolished samples the grains of the polycrystalline Ti bulk were clearly visible. An accentuation of the grain boundaries and a fine structure of some of the grains was observed after the plasma treatment. Different orientations of the Ti grains underwent different topographical modifications during the plasma treatment. The overall quantification of both c.p. Ti and Ti alloy, originally very smooth and machined, respectively, shows that initially (<2 min.) the Ar-plasma resulted in a smoothening of features of sizes in the range 10-300 nm followed by a roughening of the same features, while features of larger sizes (>1micrometer) were mostly unchanged.

Effect of surface topography on bone marrow cells in vitro

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The surface of permanent bone related implants are usually modified by sandblasting or plasma spraying to improve osseointegration. The early phase of cell-material interaction is still poorly understood in terms of cell reaction to topographical variations. On one hand these implants are intended to remain in the patient, on the other hand a quick osseointegration of the implant is desired to reduce the time of hospitalization.

Three different methods of surface modifications were applied on c.p. titanium and on it'ss alloys with the intention to improve the early phase cell performance on the material surface. Surface modifications were performed by conventional sandblasting and plasma spraying, by micro-fabricating defined structures in the range of 0 to 500 µm, and finally by chemical coupling of -RGD- containing peptides to the titanium surface.

Two cell culture models (MC3T3-E1 osteoblastic cell lineage and primary bone marrow cells of adult male rats) were used to investigate the effect of surface modifications on bone cell proliferation (DNA and total cell protein content) and cell differentiation (ALP activity of osteoblastic cells, TRAP activity of osteoclastic cells) after an incubation period of 14 days. Morphological effects were analyzed using SEM.

Results: The effects of various structures on cells were depending on the culture model. The bone marrow cell culture model seemed to be more sensitive to modified surface topography. Generally, an increased surface roughness reduced ALP activity of osteoblastic cells. Similarly, the peptide coated titanium surfaces did not improve bone marrow cell performance in contrast to the osteoblastic cell lineage.

These data suggest, that in vitro bone marrow cell differentiation is determined by other surface properties (e.g. plain surfaces) than in vivo in the later stage of ossification, where roughened surfaces seem to be preferred by calcified bone tissue.

Resorbable Porous ceramics in spinal arthrodesis.

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A new porous ceramic graft material was studied in the sheep for resorption and its potential to promote posterolateral spinal fusion. The spinal fusion outcomes were analysed and compared to the results (using similar, but non-resorbable materials) from a former study. Twenty-four skeletally mature Dorset sheep had an instrumented posterolateral fusion from L2-L4 using a 50%/50% volume mixture of Pro Osteon 5ooR (Interpore International, Irvine CA, USA) and cortico-cancellous autologous bone from the spinous processes. 7 animals each were sacrificed at 12 and 20 weeks, and 5 animals at 36 weeks postoperatively. All groups had post-mortem plain radiography, CT reconstruction, biomechanical testing and histological evaluation performed.

At 12 weeks on average 33% (0%-70%), at 20 weeks 74% (40%-90%) and at 36 weeks more than 90% (80% to 100%) of the ceramic granules were resorbed ($p<0.01$). At 12 weeks about half of the graft sites showed 50% or more bridging and at 20 weeks all but one segmental level had solid fusion with new bone cortex formation. At 36 weeks all segmental levels had solid fusion. There was no statistical difference between a 50%/50% percent volume mix of Pro-Osteon 500 R with autologous bone and autologous bone alone at 20 weeks and 36 weeks postoperatively. Histological evaluation demonstrated a 90% surface coverage at 20 weeks, and close to 100% coverage at 36 weeks postoperatively for the resorbable porous ceramic material, compared to only 65% for the non-resorbable material at 20 weeks ($p<0.01$).

The porous ceramic graft substitute used in the sheep model demonstrates between 12 and 20 weeks marked resorption and new cortical bone formation. There was no significant difference in strength for the pure autologous group and the porous ceramic substitute mixed with locally harvested bone past 20 weeks. Porous resorbable ceramic is a suitable volume enhancer of locally harvested autologous bone, possibly alleviating the need for pelvic bone harvest. In the future, autologous bone graft harvest from the iliac crest in posterior spine fusion may become the exception.

Changing views regarding, materials, procedures, function and biology in implants for temporary function.

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The use of implants in the treatment of disorders of the locomotor system of the human and animal body serves the purpose of either permanent replacement of temporary support. Permanent replacement is performed when e.g. articulations as a result of degenerative disease have degraded to a point where only replacement offers a solution to the problems of degradation of biologic function. Temporary support is needed when a bone has lost its support function mainly as a result of trauma. Similar requirements and views apply to both functions of the implant. We will discuss some aspects of such requirements using temporary support as an example.

The function of bone in context with the locomotor system is closely linked to its structural stiffness. As a result of mechanical overload bone loses its structural integrity in a violent process of separation at single or multiple locations. The net result consists in loss of stiffness that can be best understood as a discontinuity of the bone stiffness. The loss of stiffness results in load induced deformation of the tissues, such deformation occurs in a confined area and thus produces high values of strain of the soft tissues.

The process of fracture healing starts with a discontinuity of stiffness of the broken bone and should end with restoration of continuous stiffness: solid union providing adequate strength for resisting functional load. Observation of the healing process in wild animals shows that such solid union can be obtained as rule without the medical intervention. The fact that spontaneous solid union results in malalignment is the reason for the medical treatment with the goal to avoid malalignment and thus ensure proper function of the limb.

To allow for healing in proper anatomical reconstruction temporary support of the fracture fragments is required. Such support must maintain the fragments in proper anatomic relation and allow for conditions to allow for prompt healing, avoid disadvantages of early and prolonged stabilisation and in addition must ensure conditions that minimise side effects.

Prompt solid union requires biomechanically a condition of enough dynamic strain to induce the process of healing at the same time as not exceeding the tolerance of strain of the repair tissues. Thus the interface of the implant to bone is critical. Biologically the condition for prompt healing is maintenance or reconstruction of blood supply to avoid or repair necrosis. The side effects of excessive external stabilisation may be reflex dystrophy and the side effects to be avoided consist mainly in increased susceptibility to infection in the presence of so called “foreign bodies”. We presume today that provided the biomaterial is well tolerated the effect on local resistance to infection is mainly a question of the interface between implant and soft tissues. Two conditions may prevail. The first condition may consist in high strain at the interface that prevents adherence of the tissues allowing for a dead space to exist. Such dead space favours bacterial growth and spreading and at the same time reduces accessibility of the bacteria to blood supported defence. The second condition may be intimate and permanent adherence of the soft tissues to implants a condition that favours defence of bacterial infection.

Of the three conditions mentioned: function, interface to bone and interface to soft tissues the former two seem to be fairly well solved while the latter offers most potential and challenge for improvement.

Materials and Implants for Augmentation or Reconstruction of Vertebral Bodies and Intervertebral Disks

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Back pain due to micro- and macrofractures of vertebral bodies after osteoporotic destruction of the bony architecture can be prevented or repaired by vertebroplasty. For this minimal invasive surgical technique either polymethylmethacrylate (PMMA) bone cement, or so-called "bioactive" bone cements can be injected into the destructed areas. While PMMA has the advantage of lower viscosity and thus better applicability, the foreign body reaction to this classical bone cement and a possible disturbance of mineralisation in the adjacent bone are points of concern. The latter problem can be partly overcome by the addition of "bioactive" calcium-phosphate materials to PMMA. An even more promising approach are the resorbable calcium-phosphate cements which also offer sufficient mechanical stability (1), but seem to be more difficult to distribute in the affected bone. Both types of materials are also used to stabilise pedicle screw osteosynthesis in osteoporotic vertebrae.

The much more invasive surgical treatment of back pain by interbody fusion in the cervical or lumbar spine, augmented by either horizontal cylinder, or vertical ring, or open box implants, is rapidly gaining popularity. These implants are made from metallic or carbon fibre-reinforced polymeric materials, and are supplemented by autologous bone grafts and/or bone graft substitutes, in order to achieve a bony fusion of the adjacent vertebrae. From a recent review (2), the biological and biomechanical responses to these materials and the inevitable wear particles will be critically analysed, and the limit morbidity of all the systems currently in use will be pointed out.

The most difficult and thus not yet really successful approach of only augmenting or replacing the degenerated intervertebral disk will finally be reviewed. The history, theory, design and choice of materials of these implants (3) show that a lack of knowledge and understanding of the complex structure and function of these parts of the spine are the major causes of failure.

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Interfacial and peri-implant bone reactions to mechanically interlocking metallic implants

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The concept of primary mechanical interlocking is not only used for the clinically successful screw-shaped dental implants and osteosynthesis screws, but also for implants with microrough metallic surfaces where a passive frictional contact (interference and/or press fit) with the more or less congruent bone bed provides the essential primary stability. It is now the general understanding that new bone can then actively grow onto and into surface structures of any sufficiently "bioinert" metallic material. Bioinert implant materials seem to need these same appropriate macrostructures (such as screw threads, cast-on globules, or a fibre-metal coating), or microstructures (such as blasted or plasma-sprayed surfaces) for this initial woven bone ongrowth. So-called lamellar compaction of these woven bone structures bridging periimplant spaces leads then to a firm secondary mechanical interlocking with the implant and to a biologically adaptive situation suitable for functional load transfer.

Conditions for primary and secondary implant fixation will be different in a compact or spongy bone bed. Spongy bone normally exceeds compact bone in new bone formation activity, but implant insertion into an atrophic or osteoporotic spongiosa may jeopardise implant success by not providing enough primary stability, or not enough periimplant connectivity despite interfacial bone contacts. In a compact bone bed, only periimplant spaces due to incongruencies can be filled by new bone, unless osteoclastic resorption of bone damaged by implant bed preparation has made room for new bone formation. On the other hand, extensive interfacial bone contacts and thus apparent load transfer or stress shielding can be regarded responsible for structural changes observed in the periimplant cortical bone after long-term implantation. In these respects it is worth considering that remodelling of interfacial and periimplant bone, which is influenced by all kinds of interactions with the implant materials and biomechanical forces, will not only determine the initial healing-in of the implant, but also will continue during the lifetime of an implant.

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Semi-Quantitative and Quantitative methods to assess the cytotoxicity of prion inactivated muskulo-skeletal allografts :

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In-vitro cells test methods allow a rapid, reproducible, and objective evaluation of cytotoxicity and biocompatibility of human tissue grafts. An in vitro model of normal human cells was designed to test the cytocompatibility of prion inactivated allografts.

Bone fragments, fascia lata and tendons allografts from selected donors were excluded for bacterial contamination. They were cut in small pieces and washed under pulse lavage. They were treated with a proprietary sequence of chemical treatment including solvent detergents and NaOH for viruses and prion inactivation. Bones and tendons were further freeze dried and sterilised by gamma irradiation. Standard cell line (WI38 : fibroblastic cell) and specific cell lines (Neuro 2A : neuronal cells and SAOS2 : osteoblast) were selected to relate to the material application. The use of Neuro 2A cells was indicated as fascia lata allografts could be used as dura-mater substitute. Under sterile condition, fragment of bone (0,1 g/ml) or tendon (1 cm^2/ml) were immersed in cell specific culture medium with serum during 72H. Negative (PET) and Positive (Phenol) control were processed following the same extraction conditions and vehicles. Cells were incubated during 72H until confluency. Cells were then exposed to the extraction medium and observed after 24, 48 and 72H. Semi-quantitative and quantitavive evaluation were performed.

Semi-quantitative method was performed by analysis of cells confluence (by image processing and analysis software), morphology, granulation and vacuolisation.

Quantitative method assess the cells viability and metabolic activity.

First, we've used an assay based on the use of vital dye such as Neutral red (NR) . This one is based on the spectrophotometric determination of NR taken up by viable cells and stored in their lysosomes.

The second group of assays measures the action of intracellular enzymes on tetrazolium salts. These are converted by intracellular dehydrogenases to coloured formazans which may or may not be water-soluble. The MTT assay is based on the tetrazolium salt,3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT),the conversion of which produces an insoluble product.

Morphological, confluence, granulation and vacuolisation analysis did not demonstrate any change between negative control and tissue allografts extracts. These results were confirmed by quantitative evaluation with MTT and NR assay. Positive control was extremely damaged in both tests.

By definite evaluation of cell damage, this method offers a reliable quality control as it allows the detection of any cell damaging change in the tissue treatment protocol.

We recommend these in-vitro testing procedures as the first to be conducted to evaluate the cytotoxicity and biocompatibility of tissues.

Prevention of bone loss in oestrogen-deficient rats. As assessed by microcomputed tomography.

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Microcomputed tomography of high resolution was used to measure static morphometric parameters of bisphosphonate treated oestrogen-deficient rats. Matching of microtomographic data with histomorphometric data was used to optimise the threshold setting of the tomograph. The resulting bone reconstruction's closely correlated with microscopic images.

4-months-old rats were ovariectomised and treated s.c. once weekly with 0.3, 1.5, 7.5 ug/kg zoledronate or vehicle. A sham operated control group was also included. After 52 weeks, animals were killed and lumbar vertebrae dissected for assessment.

Results*:* A close and significant correlation between microtomographic and microscopic measurements was found. 2D and 3D tomographic analysis confirmed the microscopic data: Ovariectomy significantly reduced trabecular bone volume fraction, trabecular number and thickness. Conversely, trabecular separation and the bone surface/volume ratio increased. Trabecular thinning and plate-to-rod transition were also reflected by increased trabecular bone pattern factor values (TBPf). Zoledronate treatment dose-dependently reduced the effects of oestrogen depletion, with complete prevention at 1.5 ug/kg/ week s.c. TBPf values were also dose-dependently normalised by zoledronate treatment.

Microcomputed tomography is a useful method for detecting bone architectural changes in small laboratory animals The results of this study further corroborate the efficacy of low doses of zoledronate in the prevention of vertebral bone

Elastomer Coated Hip prosthesis: stress distribution and histology at the bone/implant interface

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The stem part of a total hip replacement (THR) can be fixed by means of an elastomer coating applied onto the metallic core of the stem. This fixation system is an attempt to introduce an elastic buffering effect in a cementless stem design. The elastic buffering effect is hypothesised to be similar to or even better than the buffering effect of a PMMA cement mantle. A stem prototype was developed and through an extended biocompatibility testing programme, an elastomer was selected for the coating. A finite element analysis (FEA) was performed in order to show the significant influence of the elastomer coating on the stress distribution around a loaded THR stem. For the prototype geometry, the effect of stem modulus, coating modulus and bending moment on the stress distribution were studied with three-dimensional FEA. For a simplified geometry, the influence of the rubber elasticity model was studied with two-dimensional FEA and was shown to be relevant. Histological results from an explorative trial on German shepherd dogs with hip dysplasia are available. It was observed that the bone remodelling was more favourable around an elastomer coated stem than around a massive titanium control stem. The resorption was less pronounced and the fibrous tissue showed less foreign body reaction with the elastomer coated stem. These observations were confirmed by semi-quantitative histomorphometric measurements. On selected locations at the elastomer/bone interface, bone apposition to the elastomer was observed.

Precision of high-resolution DXA-measurements of bone mineral in small animal models

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A promising approach of drug discovery involves the identification of key molecular targets, and to screen animals *in vivo*, in which specific genes have been over- or underexpressed. Moreover, small animals models can be used for testing the efficacy of these drugs. The objective of the present study was therefore to evaluate the precision of high-resolution DXA-measurements of the bone mineral content in small animal models.

We investigated 47 mice of different body weights and 10 rats, using a pDEXA Sabre (Norland/Stratec) dual energy X-ray absorptiometry scanner. The bone mineral content (BMC) and the areal bone mineral density (BMD) were examined at 4 different days in all animals.

In the adult mice (35g; n=14) the precision (RMS average CV%) was 1.7% for the BMD (3.5% for the BMC). In 2-week mice (8.8g; n=10) the values were 4.9% (12.7%), in 4-week mice (21g; n=9) 4.5% (9.7%) and in adult growth-hormone transgenic mice (60g; n=14) 3.1 % (3.9%). In the 10 adult rats the precision was 1.2% (2.5%) for the lower extremity, and 2.0 (5.7%) for the femur.

The results show that the BMD and BMC of adult mice and rats can be determined with high precision, but that the reproducibility is somewhat lower in younger animals with body weights of 20 g and less.

Determination of Bone Mineral Content at the Lumbar Spine by quantitative MRI: An Experimentally Controlled Study

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The purpose of this study was to evaluate experimentally and clinically whether a determination of bone mineral content of the lumbar spine is possible in fresh vertebral specimens and in humans by qMRI and DEXA. Bone density of ten fresh calf lumbar spinal vertebrae. Three different MRI sequences were conducted. The data acquisition was validated with a deuterium/water-reference. After image acquisition the cortical and the cancellous bone were separated surgically, wet weight and ash weight were determined. 62 patients were included in the human trial, thus resulting in 248 lumbar spinal vertebrae being analysed by qMRI and DEXA. Three different qMRI-sequences were performed (PS, SE, STIR). PS was controlled with Gadolineum. Correlation analysis was used to describe the relationship between DEXA and qMRI. In the experimental and the human setup, bone mineral mass correlated significantly with the total amount of pixels [$p<0.0001$] and with mean pixel volume [$p<0.0001$]. Administration of Gadolineum did not lead to improvement. The data achieved from this study encourages to further evaluating of qMRI. MRI in higher resolutions offers information to micro structure and trabecular connectivity. One technique combining these information's is regarded as ideal to improve the prediction of the risk of fracture.

Predictive Markers in Osteoporosis: A Review

A.W.A Baltzer, H. Koch, C. Lill, H.R. Merk

Osteoporosis is the most common bone disease of the first world. The incidence of osteporotic fractures is increasing and hip fractures are expected to inflict more than 6 million individuals world-wide by the year 2050. That's why the prediction of the onset of osteoporosis, and more important, the prediction of osteoporotic fractures remains one of the most important diagnostic problems. Markers, known to describe the activity of osseous turnover such as alkaline phosphatase, osteocalcin have been evaluated for their capacity to determine the risk of fracture. Levels of degradation markers such as pyridinoline and desoxy-pyridinoline crosslinks are known to be elevated in the urinary excretion of osteoporotic patients. The interpretation problems and predictive values of these markers for the risk of fracture are discussed.

Morrison reported that one common allelic variation of the vitamin D receptor gene accounted for up to 75% of the total genetic effect on bone mineral density. Various studies in different ethnic subsets have been performed to support, or to reject his findings. The current role of different allelic variations of the VDR gene is discussed in regard to the variety of factors known to be afflicted with osteoporotic fractures. A future management of the diagnostic procedure to predict the onset and the risk of fracture will be discussed.

The Future of Biodegradable porous scaffolds as bone substitutes and reversible muscle paralysing agents in the treatment of craniofacial skeletal defects.

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The problems surrounding hard tissue reconstruction of the face are challenging today's clinicians in profoundly new ways. The recent advents in medical imagery that allows for graphic recognition of deformities of the facial skeleton enhance our ability as clinicians to better plan procedures and forecast the morphological forms of reconstruction that will be required to restore facial appearance and function.

During the late 1970's mini titanium plate fixation was introduced into the armamentarium of the facial surgeon and this plating method revolutionised the ability to stabilise and fix the skeleton so that more precise bone grafting methods could be applied to the craniofacial skeleton. What was learnt was that this form of plate and screw fixation in the facial skeleton had the difficulties of growth retardation and migration of plates as the skeleton matured.

For many years surgeons have used sutures made from biodegradable polymers that are well tolerated by tissues and that are metabolised along normal biochemical pathways. The sugar polymers (e.g. glucose, lactose and galactose) are part of this new group of sutures and their biological application has been extended to include plates and screws for bone fixation, membranes that mimic bony separations less than a millimetre in thickness, scaffolds which reproduce the internal architecture of bone and beads with various drugs that are bound to their surfaces which can be used as embolising agents.

By variation in the physical aspects of the production of these polymers strength, hardness and solubility can be modulated to suit tissue requirements in set anatomical locations. The usual degradation of the material takes place by its uptake of water which promotes a breakdown of its polymer structure to small particles of sugar which then enters the normal biochemical pathways of metabolism to finally be excreted as carbon dioxide in water. The rate at which this occurs again can be varied based on the chemical and physical properties of the polymer.

In the neonate sheep model we have followed the use of these materials for periods up to 3 years and have observed their favourable biocompatibility together with degradation that does not retard facial growth. The exciting part of their use can be extended to the incorporation of bone promoting growth substances and other agents which influence the pattern of vascularisation of regenerating tissue. This means that the use of large bone grafts harvested from other anatomical locations in the body potentially can be made obsolete and that the significant morbidities that occur as a result of these harvests are eliminated. It also means that both the neonate and the aged facial skeleton can be better treated. This particularly applies to craniofacial deformities in children and the removal of large tumours of the face from the aged population. Consequently there will be significant cost savings as procedures can be developed in the laboratory to ultimately grow these tissue parts.

In further support of changing deformed facial structures there has been a resurgence of the use of bone distracting devices which cause underdeveloped regions of bone to be drawn apart and regain a normal morphological form. So together with the alloplastic materials and tissue promoting growth factors even greater control of morphological bone development can be achieved.

Bone morphology is primarily influenced by the physiological activity of tissues that surround it. In order to modulate muscle activity there has been the introduction of the use of drugs which will selectively dampen muscle strength. Botulinum toxin A has been specifically used to temporarily diminish individual muscle activity by combining with the motor nerve that activates muscles of the facial skeleton. Its temporary activity of approximately 16 weeks allows for the biological acceptance of graft materials into the skeleton so that the grafts are truly biologically incorporated following their insertion and are not prematurely subject to excessive stress which would have occurred had the musculature been of normal strength. This is highly relevant to any form of jaw prosthesis and further adds to the stability and longevity of such devices.

Not only does Botulinum toxin control the muscle activity but at the site of injury it provides indirectly for the control of pain and so allows for early return of normal function. Because of the specific local activity its use obviates the necessity

for potent analgesics and in the chronic pain situation reduces the potential for drug addiction. This situation again is highly relevant for jaw joint prosthetic replacement.

Towards an improved prediction of the mechanical competence of bone- density and microstructure analysis in patients and animal models

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Typical features of cancellous bone are in the sub-millimeter range, hence measuring procedures with a high spatial resolution are required. Three-dimensional quantitative computed tomography (3D-QCT) has the potential of an ideal measuring procedure. Radiation dose is of concern, however, so that special techniques are required for in vivo examinations. 3D-QCT was developed to analyse the human distal forearm with a spatial resolution of the order of 100 µm. Time serial examinations are made possible by the high precision and good accuracy at the radiation dose level of 0.8 mSv. First results of longitudinal examinations of postmenopausal women reveal surprising temporal changes of cortical and cancellous bone. Hence it is now possible to base theoretical models on real patient data and consider consequences of structural changes on the mechanical competence of bone in individual patients with large scale finite element (FE) modelling.

For spatial resolutions in the 10 µm range, 3D-MicroCT is used. It allows non-destructive measurements of bone biopsies and of small animals. The examined bone is analysed with new techniques which evaluate the complete three-dimensional complex trabecular network. Studies on tail-suspended rats show how the structural mechanisms of bone loss can be inspected in great detail. Relative bone volume shows a sharp decrease after 23 days of hind limb unloading, which is mainly caused by a thinning of the structure and only by a lesser extent by a decrease in the number of trabeculae. The imminent advent of in vivo 3D-MicroCT will soon enable to follow these changes directly in the same animal.

Stereography of Non-destructive Histotomography of Hard Tissue by Confocal Laser Scanning Microscopy

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Poster presentation

Introduction: Confocal laser scanning microscopy (CLSM) is a validated tool for the non-destructive study of hard tissues (Grötz et al.Calcified Tissue 1999) and has helped to identify early consequences of radiation therapy on dentine and bone. To elucidate possible changing in the spatial architecture of hard tissues three dimensional imaging is substantial.

Method: A CLSM in reflection mode (Leica-CLSM Aristoplan) equipped with an Ar/Kr Laser, 488nm is used. Samples: a) Osteocytes in the cortical lamellae of sound human bone blocks (n= 20). b) Odontoblast processes of sound human teeth at the dentino-enamel junction (n= 20).

All specimens were studied after embedding in methyl methacrylate resin followed by sawing.

Image Processing: A cuboid of the hard tissue is scanned obtaining a series of histotomographic images of about 60µm in the z axis. A special software (TCS NT, Leica) visualises the spatial cuboid from this staple of images. A parallax is simulated and via red/green encoding, the three dimensional image is acquired.

Results: This technique allows the identification of osteocytes embedded in the lamelar structure of the bone as known from the two dimensional CLSM images. In addition the spatial architecture of the cells with their cytoplasmatic processes and junctions are assessable. In tooth specimens the three dimensional prismatic structure of enamel, dentinal tubuli as well as odontoblast processes can be visualised.

In conclusion the technique of CLSM imaging and processing software enables the study of three dimensional objects without destruction thus minimising the risk of artefacts. It may help to elucidate the physiology and pathology of hard tissues spatial architecture.

Protein Resistance of Self-Assembled Poly(l-lysine)-g-poly(ethylene glycol) Layers on Oxide Surfaces as Measured by the Optical Waveguide Technique

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Poster presentation

A method for coating metal oxide surfaces with a PEG containing polymer has been investigated and found to greatly reduce the observed protein adsorption. Non-specific protein adsorption is a problem that plagues a wide array of biomedical applications such as implants and serum contacting sensors. In the case of implants, protein adsorption onto the implant

surface is the first stage in the series of events that leads to a deleterious immune response. In the case of sensors, protein adsorption often leads to a sensor response that is not analyte specific. Since metals such as titanium and their related surface oxides are commonly present in biomaterial applications, a method for reducing protein adsorption on oxides would be an important development in biomaterial technology.

A class of materials based on poly(ethylene glycol) (PEG), a hydrophilic polymer with many properties similar to water, has been found to be remarkably resistant to protein adsorption, and many strategies for the immobilization of PEG onto surfaces have been developed. The polymer, poly (l-lysine)-g-poly(ethylene glycol), consists of a poly(l-lysine) (PLL) backbone that has been grafted with PEG side chains. The assembly of the polymer film onto the surface is based on the electrostatic interaction of the positively charged polymer backbone and the negatively charged metal oxide surfaces. Since this immobilization relies on electrostatic interactions, pH is clearly an important parameter in such systems. The resultant surfaces have been found to exhibit drastically reduced protein adsorption. The Optical Waveguide Lightmode Spectroscopy (OWLS) technique is well suited for the study of this system.

An Online Toxicological Sensor Based on the Optical Waveguide Technique

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Poster presentation

A direct online method for measuring the morphological properties of surface adsorbed cells and thus the cellular toxicological response is described. Recently it has been shown that the kinetics of living animal cell adhesion, spreading and growth can be monitored quantitatively via the interaction of the cells with the evanescent electromagnetic field present at the surface of an optical waveguide. The quantitative information derived from the Optical Waveguide Lightmode Spectroscopy (OWLS) measurements reveals the evolution of cell-surface interaction over time. Combining Confocal Laser Scanning Microscopy (CLSM), providing information about the 3-dimensional shape of the cells at the surface, with the OWLS technique would allow for the correlation of the cell shape and function information with the cell-surface interaction measurement. Furthermore, surface adsorbed cells often respond by changing morphological properties when in the presence of a toxic substance.

Based on this principle, an on-line, in situ cell viability sensor with potential applications in toxicology, pharmacy and biocompatibility was constructed. The proposed design of the microsystem sensor involves the establishment of an osteoblast layer on the surface of the waveguide and the subsequent measurement of cellular response to various toxic substances. The prototype design of the sensor system will take into account the demand for parallel measurements in high-throughput assays.

Three-dimensional analysis of mitochondrial distribution in living osteoclasts in culture.

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Poster presentation

Osteoclasts have abundant mitochondria, which are thought to play a crucial role in the generation of energy essential for migration and bone resorption. In the present study, we investigated three-dimensional (3D) distribution of mitochondria in living osteoclasts in culture. Osteoclasts were isolated from the femora and tibiae of 0- to 2-days-old Wistar rats onto glass coverslips or bovine cortical bone slices. Osteoclasts on glass coverslips were examined within 12 h after cell attachment, and those cells on bone slices were investigated within 12 h or after 72-84 h culture. Isolated osteoclasts were loaded with rhodamine 123 as a specific fluorescent probe for mitochondria and 1.5 micro m-thick optical sections were obtained by a confocal laser scanning microscope. Their serial images were reconstructed into 3D images by an image analysing system.

Osteoclasts cultured on bone slices for 72-84 h made resorption lacunae and extended their height along the vertical axis. Osteoclasts with larger mitochondrial volume increased in number during longer culture period. There was significant correlation between number of nuclei per cell and mitochondrial distribution along the vertical axis in 72-84 h-cultured osteoclasts on bone slices. Osteoclasts isolated on glass coverslips did not show remarkable changes in their height and mitochondrial volume. These results indicate that changes of mitochondrial distribution and volume in osteoclasts are correlated with bone resorptive activity.

A Novel Combined Perfusion/loading Chamber for BONE Biomaterial Studies.

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Poster presentation

A perfusion-loading-mechanical testing device is described for trabecular bone which allows long term culture (over 60 days) without loss of viability of osteocytes or other bone cells, including adipocytes, stem cells, osteoclasts and endothelial cells. Bone morphology is essentially unaltered during this time Evidence of physiological bone remodelling and a significant increase in bone mechanical strength is present after 10 days of mechanical loading at physiological levels. The accuracy of the loading/measuring system is 0.1N and 50nm or better (less than 1% error). The new *ex vivo* system can be used to study many aspects of biomaterial interactions especially during controlled loading conditions. Measurement of the mechanical properties allows on-line monitoring of bone ingrowth. Optical on line measurements of bone physiology and morphology are also possible. Since many samples can be taken from the same animal, variation is kept to a minimum. The system offers physiological bone responses representative of in vivo combined with advanced biochemical, online morphological and genetic analysis which are normally the advantages of *in vitro*. On line mechanical properties measurement is a novel feature.