11th Essen Symposium on Biomaterials and Biomechanics: Fundamentals and Clinical Applications

of the Working Group on “Biomaterials and Tissue Compatibility”

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Final Program and Abstracts

March, 5th – 7th, 2009
University Duisburg-Essen
Campus Essen

Organizers:
Alfons Fischer, Herbert Jennissen, Dieter Bingmann

Scientific Advisory Board:
Scope of the Symposium

More than 100,000 total hip or knee joints are implanted during one year in Germany. Within a time range of ten years about 5% of those fail by aseptic loosening. The interaction of particles released from the articulating surfaces of cup and ball and the surrounding tissue is one of the major reasons. In cardiology stent related therapies have emerged into clinical application strongly since 1986. Even though many different designs, coatings, and eluted drugs have been incorporated into this treatment about 10 to 20% of the patients still suffer from restenosis. Spine discs emerge into daily clinical practice, which take advantage of the understanding of materials behaviour gained with hip implants over decades. These are just a few examples which depict the possibilities and limitations of modern medicine. The challenge of basic and clinical research is to understand the acting mechanisms and bring about measures to counter these problems by a well-aimed selection of suitable therapies. Due to the fact that this must incorporate specialists from all faculties this symposium has been established 11 years ago in order to present a forum for interdisciplinary up-to-date presentations and lively discussions.

The scopes of the 11th Symposium emphasise the properties of surfaces of biomaterials and their interaction with the surrounding soft and/or hard tissue. This implies the characterization and the functionalisation of surfaces. Special presentations on laboratory and computer simulations will be provided to show the possibilities of these new methods during basic research, product development, and clinical applications. Due to the fact that daily clinical experience renders the goals for any development it will be a substantial part of this symposium.
Program:

Thursday, March 5th, 2009

7:45  Registration

8:20  Welcome
     Alfons Fischer (Duisburg-Essen, Germany)

8:30 – 10:00  Novel Biomaterials

   Chairperson:  A. Fischer, Duisburg, Germany

8:30  Biosilica: a novel biomaterial from sponges for biomedicine and nano-Biotechnology
     W. E. Müller

9:00  Biomorphous Hydroxyapatite-Ceramics
     J. Will

9:30  Crystallization of Calcium Phosphates on Polyelectrolyte Bi- and Multilayers
     H. Milhofer

10:00  Coffee Break

10:30 – 13:00  Biomaterials and Cells

   Chairperson:  H. Milhofer, Jerusalem, Israel

10:30  Size-defined calcium phosphate nanoparticles serve as a superior tool for
       cellular DNA transfection: Absence of intracellular Ca^{2+} disturbance
       S. Neumann

11:00  Organic-Inorganic Nanocomposites for Coating Titanium Surfaces.
       S. Grohmann

11:30  Molecular-dynamics simulation of BMP-2 adsorption on a hydrophobic
       surface
       A. Oliveira

12:00  The influence of cyclic dynamic strain on the biocompatibility of cell loaded
       metallic implant materials.
       T. Habijan

12:30  Mechanical properties of blood clots - A new test method -
       N. Krasokha

13:00  Lunch
11th Essen Symposium on Biomaterials and Biomechanics: Fundamentals and Clinical Applications
March, 5th – 7th, 2009, University Duisburg-Essen, Campus Essen

14:00 – 17:00  **Biomaterials and their Interaction with Cells and Tissue**

Chairperson:  **D. Büsselberg, El Paso TX, USA**

14:00  *Lanthanides to trace wear debris of polymers in vivo*
M. Wimmer

14:30  *In Vitro Response of Periprosthetic Cells to Lanthanides*
P. Pennekamp

15:00  *Profound differences in biocompatibility of two very similar Rare-earth containing Mg-alloys*
J. Reifenrath

15:30  **Coffee Break**

Chairperson:  **D. Bingmann, Münster, Germany**

16:00  *Toxicity of Metals*
D. Büsselberg

16:30  *Effects of respirable particles on lung tissue: Comparison of in vitro and in vivo testing*
M. Wiemann

17:00  **Postersession and Snacks**
14:00 – 17:00 **Computational Methods for Preoperative Planning**

Chairperson: **W. Kowalczyk, Duisburg, Germany**

14:00 *Artefact reduction in marker-based gait analysis by integration of MRI data.*
A. Kecskemethy

14:30 *Segmentation of Muscle Sartorius with Tendon Attachment Sites from Magnetic Resonance Images*
J. Pauli

15:00 *Simulation of biodegradable implants for bone replacement with a multiphase theory.*
T. Ricken

15:30 **Coffee Break**

Chairperson: **A. Kecskemethy, Duisburg, Germany**

16:00 *Application of PCA and RTG Images for 3D Reconstruction Geometry of the Bones*
M. Rychlik

16:30 *Pre-surgery planning in Orthopedics by means of Medical Rapid Prototyping*
T. Mallepree

17:00 **Postersession and Refreshments**
Friday, March 6th, 2009

7:45  **Registration**

8:30 – 11:30  **Composites with BMP-2**

Chairperson:  **M. Laub, Essen, Germany**

8:30  *Manufacturing of novel materials for BMP coupling process*

K. Koczur

9:00  *Bone morphogenetic proteins 2, 5 and 6 in combination stimulate osteoblasts but not osteoclasts in vitro*

A. Wutzl

9:30  *BMP at the Solid-Liquid Interface*

H. Jennissen

10:00  **Coffee Break**

Chairperson:  **H. Jennissen, Essen, Germany**

10:30  *BMP-2 Loaded Microstructured Phycogenic Bone Substitutes*

K. Zurlinden

10:45  *Biocoating of Hydroxyapatite of Phyogenic Origin: In Vivo and Clinical Results*

R. Ewers

11:15  *Accelerated Hydrolysis of Foamed rhBMP2/Poly-(D,L)-Lactide*

T. Sänger

11:30 – 13:00  **Hard Ceramic Couples in Orthopedics**

Chairperson:  **M. Wimmer, Chicago IL, USA**

11:30  *The Tribology of Squeaky Hip Joints*

M. Morlock

12:00  *Wear Mechanisms on a Squeaky Alumina-on-Alumina Hip Prosthesis -A Case Report-

R. Pourzal

12:30  *Alumina matrix composites for Arthroplasty*

T. Pandorf

13:00  **Lunch**
14:00 – 17:00  **Particles in Hard Metal-on-Metal Bearings**

Chairperson: **J. Medley, Waterloo, Canada**

14:00  *Microstructural Alterations of CoCrMo-Alloys under Articulation. Experiment and Computer Simulation.*

A. Fischer

14:30  *Microstructure and Surface Characterization of Metal-on-Metal Hip Joints*

M. Spinelli

15:00  *Characterization of Particles and their effect on Cells*

I. Catelas

15:30  **Coffee Break**

Chairperson: **I. Catelas, Ottawa, Canada**

16:00  *MoM Tribology - Reason for Particles*

J. Medley

16:30  *MoM - The clinical point of view*

P. Beaule

17:00  **Postersession, Refreshments and Snacks**
Saturday, March 7th, 2009

7:45  **Registration**

8:30 – 10:00  **Materials and Methods in Trauma Surgery**

Chairperson:  **M. Epple, Essen, Germany**

8:30  *Clinical Relevance of Corrosion of Thin Layers in Joint Arthroplasty.*
G. Taeger

9:00  *Does Corrosion Matter in Titanium Plates?*
B. Hußmann

9:30  *A next generation of implant material or just another piece of metal: Summary of experimental data of P2000.*
M. Weuster

10:00  **Coffee Break**

10:30 – 13:00  **Materials and Methods in Trauma Surgery and Orthopedics**

Chairperson:  **F. Löer, Essen, Germany**

10:30  *Modular adaptive bone plate based on intelligent materials.*
D. Tarnita

11:00  *Porous Metals in Orthopedic Applications*
B. Levine

11:30  *Wear of contemporary knee designs and materials*
S. Utzschneider

12:00  *DNA-repair mechanisms in Aseptic Loosening after Total Hip Replacement*
S. Landgraeber

12:30  **Farwell Snack**
Session: **Novel Biomaterials**

in presentation order:

W. E. Müller, Mainz, Germany  
*Biosilica: a novel biomaterial from sponges for biomedicine and nano-biotechnology*

J. Will, Erlangen, Germany  
*Biomorphous Hydroxyapatite-Ceramics*

H. Milhofer, Jerusalem, Israel  
*Crystallization of Calcium Phosphates on Polyelectrolyte Bi- and Multilayers*
Nature represents a seemingly inexhaustible source of inspiration for biotechnological approaches in order to meet the ever-increasing demand for novel composite materials with unique functionalities. Biological hard tissues are prime examples of hybrid composites that exhibit excellent physical properties and comprise both inorganic and organic molecules. Based upon these templates the emerging field of nanotechnology facilitates the design of biomimetic nanoscale composites that display significant advantages over existing composites thus far used in biomedicine/biotechnology. Industrial processing of siliceous materials is not only restricted to the production of glass objects of daily use but also is silica the raw material for biotechnological high-tech products in the fields of biomedical engineering and regenerative medicine. However, the processing requires harsh conditions, consequently destroying sensitive materials that otherwise might be used in combination with silica to generate novel materials. Nevertheless, silica structures are also synthesized in living organisms: siliceous sponges. Of all recent animals only sponges are able to polymerize silica to obtain macroscale skeletal elements (spicules) during a unique reaction, at ambient temperature and pressure. Through genetic engineering the poriferan enzymes that mediate not only the formation but also the degradation of biosilica (termed silicatein and silicase respectively) were expressed and characterized [1-3]. Since then many efforts (based on in vivo-, in vitro-, and in silico- approaches) were directed towards understanding the molecular mechanisms that involve silica-enzymes. Thus, it was found that silicatein catalyzes the in vitro synthesis of amorphous silica from anthropogenic organosilicon compounds via an esterase activity [4]. However, silicatein not only catalyzes the formation of interconnected silica nanospheres but also mediates the synthesis of titania (TiO₂) and zirconia (ZrO₂) from respective monomers [2,3]. These results indicate a rather relaxed substrate specificity that might be further exploited with respect to a broader range of silicatein substrates. In addition, molecular modeling proposed the 3D-structure of silicatein and its catalytic conformation, posttranslational protein modifications were predicted, and enzyme kinetics determined [4,5]. Furthermore, in the course of conceptual developments silicatein was immobilized on surface-functionalized TiO₂ nanowires. As a reductant silicatein then synthesized Au nanocrystals from an AuCl₄⁻ source, resulting in TiO₂/Au nanocomposites [6]. Hybrid materials that consist of nanoparticles attached to nanowires have attracted attention since they combine unique characteristics with magnetic, electronic, and structural properties [6]. However, the majority of hybrid materials so far generated comprise nanoscale carbon structures. Therefore, TiO₂/Au nanocomposites open a new horizon towards applications in the field of biomaterial science. On the other hand the biomedical potential of silicatein was highlighted by the surprising finding that biosilica stimulates the mineralization activity and protein expression in osteoblast and ameloblast model systems [7]. In conclusion, recombinant poriferan silica-enzymes are of high interest for a variety of biomedical and biotechnological applications, ranging from surface modifications in nanobiotechnology to novel materials for bone replacement and dentistry or coating of metal implants, all procedures that would benefit from the advantages of the original biological system.

References:


enzymes from sponges: basic aspects and application in nanobiotechnology (material sciences and medicine). Naturwissenschaften 94, 339-359.


NOTES:
In nature, the astonishing biomechanical properties of tissues such as bone and ligament depend on their hierarchic morphology. Hierarchical structures are assemblages of structural units, organized at increasing size levels. Such multilevel architectures are capable of conferring unique properties to the whole structure. In recent years, a novel replication technology, i.e. biotemplating based on the conversion of wood into a variety of porous biomorphous ceramics has been established. In the transformation process natural wood is pyrolysed to obtain carbon templates that are infiltrated with metal containing melt or vapour and subsequently reacted to form carbide phases (SiC, TiC). In the substitution process, native or pyrolysed wood templates are infiltrated with salts or metal organic precursors and subjected to oxidation to remove the carbon afterwards.

This study focuses on the process transforming the unique hierarchical structure of rattan plants into hydroxylapatite (HA) designed for bone substitution via a sol-gel process. Native rattan (Calamus rotang) belonging to the group of monocotyledon wood possesses no growing rings and shows large pores with a diameter up to 600 µm corresponding to the large metaxylem vessels as well as smaller pores up to 30 µm belonging to metaphloem vessels.

Rattan samples were impregnated under vacuum using a sol consisting of triethyl phosphite and calcium nitrate in an ethanol-based solution. Infiltration steps were repeated up to three times. After each infiltration step gelation was carried out in a drying furnace at 80°C. The impregnated, dried rattan was then pyrolysed under N2-atmosphere at 800°C to obtain a biocarbon structure. The sol-gel process is repeated up to three times using this biocarbon structure as template that was removed afterwards via a heat treatment in air and the hydroxyapatite was sintered in air at 1200°C.

The inherent pore structure of the rattan template was perfectly transferred to the final HA ceramics. Pore size distribution measurements and image analysis show a three-modal pore size distribution with large pores of about 300 µm. The FT-IR and XRD analysis shows a dominant phase of HA. The hierarchical structure of the HA results in high compression strengths of 3.45±0.74 MPa in grow direction of rattan, 1.93±0.85 MPa in the radial direction of the rattan and porosities of 70-80%. As a summary, Fig. 1 shows a photograph of the three processing steps: native rattan, biocarbon template (with HA impregnated pyrolysed rattan) and sintered biomorphous HA-ceramics.

Fig. 1: Photograph of rattan, carbon template and biomorphous HA-ceramics.
CRYSTALLIZATION OF CALCIUM PHOSPHATES ON POLYELECTROLYTE BI – AND MULTILAYERS

P. Bar Yosef Ofir, H. Füredi Milhofer and N. Garti

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Introduction: There is considerable interest in organic-inorganic composites with calcium phosphate crystals as the dispersed inorganic phase, the latter having been shown to enhance bioactivity and facilitate osteointegration. Such materials may be used as bioactive coatings to facilitate osteointegration of artificial implants (metals and/or polymers) which are bioinert but, because of their mechanical properties, are utilized for the repair of large defects in the skeleton. Recently we described novel organic/inorganic nano-composite coatings, obtained by growing calcium phosphate crystals "in situ" onto and within self-assembled polyelectrolyte multilayer (PE ML) films, which consisted of alternately adsorbed layers of poly-L-lysine (PLL) and poly-L-glutamic acid (PGA) [1,2]. PE ML films of the above polyelectrolytes have been thoroughly characterized because of their biocompatibility and biodegradability [3, 4]. However, although the organic matrix described above functioned as a structural scaffold, it did not facilitate calcium phosphate crystallization. In this work we investigated a number of alternative, self-assembled bi- and multilayers in order to assess their effect on "in situ" calcium phosphate crystallization.

Materials and Methods: The bi- and multilayers (Table 1) were deposited onto glass plates (microscope slides cut to size) by the dipping method first described by Decher [5]. The modified surfaces were subjected to one of the following calcifying procedures: (a) direct crystallization of calcium hydrogenphosphate dihydrate, DCPD, from metastable solutions either in 0.3 M NaCl or in MES-Tris buffer, pH 5.5 (calcifying solution I), (b) crystallization of calcium deficient apatite by a two step procedure [1], i.e. first depositing amorphous calcium phosphate, ACP upon a PE ML and then immersing the samples for 48h into a metastable calcifying solution adjusted to pH 7.4 (calcifying solution II). DCPD crystals were observed by optical microscope, while calcium deficient apatites were visualized by scanning electron microscope (SEM) and their composition determined by electron dispersion X-ray spectra (EDX).

Results: (a) Direct crystallization of DCPD was induced on PEI monolayers and on bilayers 1 – 4 listed in Table 1. In all systems typical plate-like crystals, with dominant (010) planes were obtained. Precipitates were polydispersed, with large crystals ranging to about 10 x 50 µm.

(b) Crystallization by two-step procedure. Upon clean glass plates, which were subjected to the two-step procedure (first deposition of ACP, afterwards immersion into MSS, ref. [1]) significantly fewer crystals were deposited than on any of the substrates covered with polyelectrolyte bi – or multilayers; showing that the deposition of PE layers is essential for growing calcium phosphate crystals. The influence of the PE ML composition was striking: by far the highest number of crystals was formed on plates covered with PEI/phosvitin (bilayer 4).

A similar effect has been demonstrated by Addadi et al, who demonstrated oriented growth of calcium carbonate crystals on surfaces of sulfonated polystyrene, on which PA8p was adsorbed [6].
Table 1. Composition of bi- and multilayers tested

<table>
<thead>
<tr>
<th>Bi-layers</th>
<th>Multilayers</th>
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<tbody>
<tr>
<td>1. PEI / PSS</td>
<td>5. PEI / PSS / PAsp</td>
</tr>
<tr>
<td>2. PEI /PGA</td>
<td>6. (PLL / PV)₄</td>
</tr>
<tr>
<td>3. PEI /PAsp</td>
<td>7. (PLL / PGA)₄</td>
</tr>
<tr>
<td>4. PEI / PV</td>
<td>8. (PLL / PV)₅</td>
</tr>
<tr>
<td>9. (PLL / PAsp)₅</td>
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</table>


Conclusions: The above results show essential differences in the efficiency of different negatively charged polyelectrolytes in inducing calcium phosphate crystallization on surfaces. The efficiency of anionic polyelectrolytes increases in the order: PGA < PAsp << PV. Clearly PV, a protein containing arrays of phosphate functional groups is by far the most efficient. In addition, a combination of highly charged sulfonate groups (stemming from PSS) with carboxylate groups (from PAsp) seems to be more efficient than either one of those polyelectrolytes alone. Considering our model systems these phenomena might be explained by more efficient adsorption of the precursors (nuclei of DCPD crystals and/or ACP particles) and/or by the more efficient polyelectrolytes acting as additional nucleators.

References:


Acknowledgement: The financial support by the German-Israeli Foundation (GIF) is gratefully acknowledged.

NOTES:
Session: **Biomaterials and Cells**

in presentation order:

S. **Neumann**, Bochum, Germany  
*Size-defined calcium phosphate nanoparticles serve as a superior tool for cellular DNA transfection: Absence of intracellular Ca\(^{2+}\) disturbance*

S. **Grohmann**, Heiligenstadt, Germany  
*Organic-Inorganic Nanocomposites for Coating Titanium Surfaces.*

A. **Oliveira**, Dresden, Germany  
*Molecular-dynamics simulation of BMP-2 adsorption on a hydrophobic surface*

T. **Habijan**, Bochum, Germany  
*The influence of cyclic dynamic strain on the biocompatibility of cell loaded metallic implant materials.*

N. **Krasokha**, Bochum, Germany  
*Mechanical properties of blood clots - A new test method –*
Size-defined calcium phosphate nanoparticles serve as a superior tool for cellular DNA transfection: Absence of intracellular Ca\(^{2+}\) disturbance

Sebastian Neumann\(^1\), Anna Kovtun\(^2\), Irmgard D. Dietzel-Meyer\(^1\), Matthias Epple\(^2\), Rolf Heumann\(^1\)

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Gene therapy suffers from a low efficiency of gene transfection rates unless viral vectors are used which carry an inherent risk of causing inflammatory reactions or cellular transformation by insertion mutagenesis. In principle, the standard calcium phosphate transfection method might be suitable in vivo because of the low toxicity of Ca\(^{2+}\) or phosphate ions. By tracing added calcium by using the radioactive isotope \(^{45}\)Ca\(^{2+}\) in the human bladder carcinoma cell line T24, we show that the overall calcium uptake is enhanced during the standard calcium phosphate transfection method. By using the intracellular Ca\(^{2+}\)-indicator fura-2, we demonstrate by time-resolved imaging experiments that there are dramatic transient changes of intracellular free Ca\(^{2+}\) levels during the standard calcium phosphate transfection method. These deregulated intracellular Ca\(^{2+}\) levels are associated with an enhanced rate of cell death. These results are in accordance with our previous data suggesting that calcified deposits could destabilize atherosclerotic plaques by initiating inflammation and vascular smooth muscle cell death (Ewence et al., 2008). In order to circumvent adverse effects by calcium phosphate nanocrystals we have developed a transfection technique which employs biocompatible multishell calcium phosphate nanoparticles functionalized with nucleic acids (Sokolova et al., 2006). These size-defined calcium phosphate nanoparticles showed virtually no disturbances in intracellular Ca\(^{2+}\)-levels and cell survival rates were within the limits of untreated control cultures. In conclusion, due to their biocompatibility DNA-functionalised calcium phosphate nanoparticles may serve as a superior transfection agent for gene therapy and DNA vaccination in future.

References:

2. Ewence AE, Bootman M, Roderick HL, Skepper JN, McCarthy G, Epple M, Neumann M,
ORGANIC-INORGANIC NANOCOMPOSITES FOR COATING TITANIUM SURFACES

Steffi Grohmann, Susanne Eisenhuth, Holger Rothe, Klaus Liefeith

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Introduction: Steadily growing multilayer films can be constructed through the layer by layer adsorption of oppositely charged polyelectrolytes (PEM films). Especially for applications in the field of implantology biocompatible polyelectrolytes like poly-L-lysine (PLL) and poly-L-glutamic acid (PGA) display a high potential. In order to optimize PEM films for medical applications; i.e. coating of implants for hard tissue repair; both growth factors (like bone morphogenic protein 2, bmp-2) and calcium phosphates can be incorporated into the organic matrix. The resulting bioactive organic-inorganic nanocomposites possess the potential for an osteoinductive stimulation of bone progenitor cells.

Results: With zeta potential measurements the surface charge after each deposition step was determined to change from +40 mV to -70 mV for PLL and PGA, respectively. Optical measurement techniques like reflectometric interference spectroscopy on biochips and ellipsometry in flow chambers allowed for a time resolved analysis of the film construction on different substrates. The resulting film thicknesses correlate well with atomic force microscopical measurements of the PEM films. Furthermore, the influence of the composition of the polyelectrolyte top layer (last layer PLL or PGA) and the effect of chemical crosslinking on the cell proliferation and spreading were evaluated. Cells were seeded onto glass plates coated with 10 double layers of PLL/PGA. No significant differences in cell vitality (95 % to 100%) on the generated surfaces were determined after 7 days, but the cell number increased by > 10% on crosslinked coatings. Cells displayed a well spread phenotype on most surfaces, only on films with a top PLL layer a significant increase in the number of rounded cells was observed. Two different buffer systems at three pH values were tested for their potential of mediating bmp-2 adsorption onto PEM films.

Discussion: Coating of titanium surfaces with biocompatible polyelectrolyte films could be demonstrated. Furthermore we could show the successful incorporation of the growth factor bmp-2 into the PEM film. Future research will be focussed on the potential bioactive effects of the thus generated substrates onto bone cells.

The buffers containing both borate and sodium dodecyl sulphate (SDS) resulted in irreproducible changes in surface charge and film thickness. When adsorbed from sodium acetate buffers at pH 4.5 onto negatively charged PEM surfaces (PGA), a reproducible increase in film thickness demonstrated a successful bmp-2 deposition. Subsequent film construction after embedding bmp-2 was not hampered. Furthermore, the incorporation could also be proved through an increase in surface charge after bmp-2 adsorption.

Discussion: Coating of titanium surfaces with biocompatible polyelectrolyte films could be demonstrated. Furthermore we could show the successful incorporation of the growth factor bmp-2 into the PEM film. Future research will be focussed on the potential bioactive effects of the thus generated substrates onto bone cells.

Fig. 1: Cytoskeleton of SAOS-2 cells grown on PEM films double stained with phallolidin (greenactin skeleton) and propidium iodide (red-nucleus).
Bone morphogenetic protein-2 (BMP-2) is known to induce bone and cartilage growth, and has shown positive results in clinical trials for treating bone defects. However, the use of BMP-2 alone is limited. Therefore, one of the current aims in the field of biomaterials is to produce prostheses and implants covered with BMP-2, which would improve the healing process. On the one hand, the BMP-2 has to be adsorbed on the implant material in a stable way, so that the surface recovery can be controlled. On the other hand, BMP-2 has to remain bioavailable, which means it has to be remobilized once the implant is placed in its target. Finding an efficient way to make stable, yet bioactive, BMP-2 coatings for prostheses and implant materials poses a difficult challenge, which requires a good understanding of the BMP-2 adsorption mechanism. In this work, the adsorption of BMP-2 on a hydrophobic surface is studied by molecular-dynamics simulations performed with the program NAMD 1 and CHARMM27 force-field 2. The crystallographic coordinates of BMP-2 3, obtained from the Protein Data Bank 4 (code 3BMP), were used for the starting geometry. The simulations were performed using the dimeric form of BMP-2, as this is the biologically active form. The hydrophobic surface was modelled by using an array of equidistant phospholipids. The distance between phospholipids was determined based on the morphology of an ideal hydroxyapatite (001) surface, and the positions of the phosphonic groups were held fixed during the simulation.

References:

Nickel-titanium shape memory alloys (NiTi) are of biomedical interest due to an unusual range of pure elastic deformability (pseudo-elasticity) with an elastic modulus (28 GPa) closer to that of bone (0,3-20 GPa) than any other metallic or ceramic material. Thus the application of NiTi as an orthopedic implant material may reduce the stress-shielding effect. However, there are still concerns because of the high nickel content (50%at%), which may result in adverse tissue reactions especially with long-term implants under mechanical strain. Nickel and titanium of NiTi-SMA are distributed in a regular crystal lattice order exhibiting high atomic bonding forces thus, it is unlikely for nickel to be released from the bulk material. In addition, the surface of NiTi-SMA is well passivated by a titanium dioxide layer which is responsible for the good corrosion resistance of this material and acts as an effective barrier to prevent nickel ion release. The integrity of the outermost surface layer is of crucial importance for the biocompatibility of NiTi-SMA. However, orthopaedic implants in the body are frequently exposed to loading/unloading conditions, being deformed in an elastic manner. In contrast, biocompatibility studies are often performed under static conditions. Mechanical stress may aggravate metallic ion release resulting in a degradation of both the body tissue and implant.

To analyze the biocompatibility of mechanically strained NiTi-SMA, tensile specimens were preloaded with human mesenchymal stem cells (hMSCs). The specimens were transferred to a sterile poly-tetrafluoroethylene (PTFE) cell culture tube equipped with a cell culture perfusion system and fixed to the pull rods of a tensile testing machine. The cell culture tube was placed into a conventional cell culture incubator located within the tensile testing machine. 86,400 strain cycles were performed for a period of 24 h and 7 d. Subsequently, the cell culture tube was disconnected from the tensile testing machine and transferred to a cell culture hood. The cell culture medium was aspirated and stored at -80°C. Interleukin-6 and nickel ion release were determined. The hMSCs on the tensile specimen were stained by calcein-AM and propidium iodine to analyze cell viability. As was shown the dynamic loading under the used conditions did not influence the biocompatibility of NiTi-SMA. However, the release of IL-6 from hMSCs was significantly higher after mechanical load compared to static conditions. Furthermore, the cycles of loading and unloading increased the nickel ion release from the tensile specimen.

The presented experimental approach will provide information on the biocompatibility and fatigue behavior of metallic specimens using sample size and dynamic strain relevant for orthopedic implants. We successfully established a cell culture perfusion system which provides nearly in vivo conditions for the culture and testing of living cells onto tensile specimens, bridging the gap between static in vitro cell culture experiments and in vivo animal experiments.

Acknowledgement: This work was supported by a grant of the Deutsche Forschungsgemeinschaft (SFB 459: Shape Memory Technique).
Mechanical properties of blood clots - A new test method -

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KEYWORDS: Clot, Stroke, Mechanical Recanalisation, Mechanical Thrombectomy, Blood thrombus,

A blood clot needs to have the right degree of mechanical, chemical and biological properties to stem the flow of blood and yet to be suitable for lytic enzymes or mechanical thrombectomy so as not to form a thrombotic event. The origin and understanding of these mechanical properties are still unknown. Clots are made of a three-dimensional network of fibrin fibers stabilized through ligation with a transglutaminase, factor XIIIa. New methods to achieve information about the in-situ mechanical properties were established. We performed compressive strength experiments of aged human blood clots. After the set up of a new test environment, we were able to perform in-situ tensile strength measurements of aged animal and human blood clots. Stress strain curves of aged clots were measured and discussed. The viscoelastic properties of the clot material were quantitatively described. This work should finally give a better understanding of the behaviour of aged blood thrombus and induced mechanical stress.
Session: **Biomaterials and their Interaction with Cells and Tissue**

in presentation order:

M. **Wimmer**, Chicago IL, USA  
*Lanthanides to trace wear debris of polymers in vivo*

P. **Pennekamp**, Chicago IL, USA  
*In Vitro Response of Periprosthetic Cells to Lanthanides*

J. **Reifenrath**, Hannover, Germany  
*Profound differences in biocompatibility of two very similar Rare-earth containing Mg-alloys*

D. **Büsselberg**, El Paso TX, USA  
*Toxicity of Metals*

M. **Wiemann**, Marl, Germany  
*Effects of respirable particles on lung tissue: Comparison of in vitro and in vivo testing*
Lanthanides to trace wear debris of polymers in vivo

M.A. Wimmer¹, J. Kunze², V. Ngai¹, L. Gallardo¹, M.P. Laurent¹, J.J. Jacobs¹

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Introduction: Wear measurements are a standard procedure to detect machinery failure before the point of collateral damage. In order to prevent machine failure, service intervals can be relatively short in comparison with the live expectancy of the wearing parts. In the case of oil lubricated metal bearings, wear can be monitored semi-continuously using different spectrometry methods (i.e. mass spectrometry). Here, the metal ion concentration within the lubricating fluid is determined. This quantitative technology is not available for polymers since their constituents are similar to the lubricating fluid and/or occur in large quantities in the environment and are thus not traceable.

In total joint arthroplasty, polymers are routinely paired with metal and/or ceramic counterfaces to treat damaged joints in the human body. Once implanted it is nearly impossible to identify the wear rate of the specific device because polymers are invisible on x-rays and the surgeon cannot simply retrieve the implant for wear analysis. Hence, the polymer bearing surface will be retrieved after complete failure of the device has occurred.

Objective: The purpose of this study is to develop markers that could be used to monitor the in situ wear performance of an UHMWPE component. Specifically, we are addressing the wear of ultra-high molecular weight polyethylene (UHMWPE) which is the most common bearing material in orthopedic use. The polymer is doped using lanthanides because of their specificity and sensitivity using mass spectrometry techniques. Using different lanthanides, wear of articulating and non-articulating surfaces (e.g. the implant backside of modular devices) could be discriminated.

Another objective is the evaluation of using lanthanide compounds to increase the resistance of UHMWPE to oxidative degradation, which is currently in its pilot phase.

Methods: To evaluate europium (Eu) and gadolinium (Gd) as a tracer materials to quantify UHMWPE wear of total knee replacement (TKR), six pins were machined from UHMWPE doped with Eu-stearate and subjected to 5mm x 5mm square motion pin-on-disk (POD) testing. The experiment was conducted to 2.1 million cycles (mc) and samples of the testing lubricant were obtained at regular intervals along with gravimetric pin measurements. The concentration of Eu and Gd was quantified in all samples, which was compared with the corresponding gravimetric measurement.

To apply the method to modular prosthetic TKR components, nascent GUR 1050 UHMWPE powder was then doped with Eu or Gd at concentrations of 49 ppm and 68 ppm, respectively. This powder was strategically placed into the wear zones, namely the bottom and the top of the tibial plateau. Direct molding was employed to manufacture the polyethylene inserts. Using a four-station knee simulator, wear was investigated following ISO 14243-1. Three test components, 1 load-soak and 1 free swelling component were tested for 5 million cycles (Mc). To track the progression of wear, the test was interrupted every 0.5 Mc and lubricant samples were pipetted from each station to measure the tracer content with mass spectrometry. In addition, the change in weight of each component was monitored.

Results: The detection limit was 2.2 ppt for Eu and 2.9 ppt for Gd (or 0.027 and 0.026 mg of PE wear). The chemically and gravimetrically determined wear during POD testing yielded a highly significant correlation ($R^2=0.955$, $p<0.001$). The slope of the least-squares regression line was not significantly different from 1.

Also in the case of TKR, the determined total (back- and topside) wear compared well with gravimetrically calculated wear after soak correction. In general, the wear rates during this test were low and averaged 1.5mg/Mc. At any point throughout the experiment, backside wear contributed no more than 10% to the total wear and averaged fairly constant at 6% after 3Mc. The free swelling and load-soak components did not show evidence of tracer dilution through chemical determination but gained weight (0.89 vs. 1.10mg/Mc) instead.

Discussion: It was possible to determine wear utilizing a novel tracer technology, despite the fact that in vitro wear rates were low. The wear performance of artificial knee
joints could be monitored and would allow the surgeon to intervene earlier and more effectively in the event of unusual component degradation. Further, it is our hope that this technology could lead to prosthetic joint implants with enhanced fatigue strength and delamination resistance, thereby increasing the service life of the implants. These improvements would particularly impact knee prostheses, currently the largest and fastest growing prosthetic joint segment in the U.S.

In summary, lanthanides used as tracer are a promising approach to quantify the amount and distribution of polymer debris in joint arthroplasty. Specific in vitro and in vivo biocompatibility tests have to follow to investigate the full potential of these materials.

NOTES:
In Vitro Response of Periprosthetic Cells to Lanthanides

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Introduction: Lanthanides are a unique group of metallic elements with several industrial and agricultural applications. Due to their favourable biochemical and pharmacological properties, some lanthanide compounds have been used in the medical field (e.g. gadolinium as contrast agent for MRI imaging). Lanthanides are also becoming attractive in the medical engineering sector where lanthanum [La3+] has been proposed to improve the machining process of titanium alloys (1). Further, a polymer doping technique was developed using europium [Eu3+] to track polyethylene wear (2,3). The biological reactions to ion release from lanthanide-treated orthopaedic implants during wear and corrosion therefore needs to be considered. Since the ionic radii decrease through the period ("lanthanide contraction"), their behaviour may be tied to the atomic number. Macrophages are known to be one of the primary cell populations in periprosthetic tissues whereas osteoblasts are essential for osseous implant integration. Due to the established chemical similarity, it was hypothesized that these elements will induce a similar response to the respective cell types.

Methods: A human osteoblast cell line (MG-63) and a murine macrophage cell line (J774.A1) were used to study the cell responses towards lanthanides. The tested lanthanide trivalent cations had low (cerium; [Ce3+]), medium (europium; [Eu3+], gadolinium; [Gd3+]), and high (lutetium; [Lu3+]) atomic numbers, and were administered as soluble chloride salts. Each cell line was incubated with increasing concentrations of lanthanide cations (0.05, 0.1, 0.5, 1, 2.5, and 5 mM) for 48h and compared with untreated cells. The effects on cell proliferation were measured using the MTS colorimetric assay. Cell apoptosis was determined by Annexin V-PE/7-AAD fluorescent double-staining of the cells using flow cytometry. Cell culture supernatants were collected for detection of pro-inflammatory cytokines using ELISA. Differences to untreated control were evaluated using Student’s t-test.

Results: Up to 1mM, the exposure of lanthanides to human osteoblasts induced no significant effect on cell proliferation and on the rate of apoptotic cell death. (Fig. 1,2). Only the highest concentrations (2.5 and 5 mM) caused a marked decrease in cell proliferation along with an increase of apoptosis. This was observed for all tested elements. Regarding the macrophage behavior, however, both Ce3+ and Lu3+ induced a significant decrease in cell proliferation at 0.05 mM (p<0.001) and 0.1 mM (p<0.0001), followed by an increase with a maximum at 0.5 mM for Ce3+ (about 95% of control) and at 1 mM for Lu3+ (about 75% of control) (Fig.1). Eu3+ and Gd3+ displayed no significant adverse effects on macrophage proliferation up to 1mM and induced considerable apoptosis initially at 1 mM and 0.5 mM, respectively. Compared to control levels, lower lanthanide concentrations did not significantly increase the TNF-alpha release which was even diminished about 50% at 1 mM (Fig.3).

Discussion: The induced cell response by various lanthanides was similar for osteoblasts but element specific for macrophages. Osteoblast proliferation and apoptosis remained unaffected up to 1mM regardless of the element. Similar cytocompatibility studies with MG-63 osteoblasts using cobalt cations (Co2+) revealed a marked decrease in cell viability already at 0.2 mM (4). In contrast to osteoblasts, macrophages showed a biphasic response for Ce3+ and Lu3+. Similar effects have been reported in an earlier study with La3+ (1). Lanthanides with lower atomic number, such as La3+ and Ce3+, have the ability to replace calcium in enzymes and other calcium-dependent processes (5) which may have altered the macrophage homeostasis.
Interestingly, macrophage mortality, which increased rapidly after exposure to Ce$^{3+}$, was mostly of apoptotic nature, thus limiting the inflammatory reaction. The early macrophage response towards Lu$^{3+}$ may be attributed to distinct chemical characteristics of heavy lanthanides, such as greater acidity and inferior solubility in fluids leading to pH-changes and metal precipitation in cell culture media. Up to 1 mM, TNF-alpha levels secreted by macrophages remained low or even significantly reduced compared to controls for all tested elements supporting reported anti-inflammatory properties of lanthanide compounds (5).

In conclusion, for phagocytotic cell types, our results reveal that lanthanides induce divergent, dose-dependent effects, which are not a function of the ionic radius.

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PROFOUND DIFFERENCES IN BIOCOMPATIBILITY OF TWO VERY SIMILAR RARE-EARTH CONTAINING MG-ALLOYS

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Introduction: Biodegradable magnesium alloys have gained much interest in recent years. These alloys offer advantages to conventional biomaterials, especially in orthopedic surgery. Biodegradation of the implants supersedes a second operation for implant removal. Adjacent to an elastic modulus close to cortical bone which reduces stress shielding, magnesium alloys enhance osteoblastic activity around the degrading implant. Rare-earth containing Mg-alloys were tested as non-allergic (1), biocompatible and slow degrading, without radiographically observable gas formation (2), which is seen in fast degrading alloys (3). The problem in producing Rare-earth containing implants is the reproducibility of the alloy composition (4) because commercially available Rare-earth compounds vary in the mixture. Due to the fact that LAE442 is a very promising implant material for orthopaedic use, the aim of this study was to replace the Rare-earth composition metal by cerium as one of the Rare-earth elements to ensure reproducibility of the implants.

Materials and Methods: The employed extruded 2.5 x 25 mm cylindrical implants were made of a magnesium alloy with 4wt% lithium, 4wt% aluminium and 2wt% cerium (LACer 442) or 2wt% Rare-earth compound (LAE442). Five New Zealand White Rabbits were used in each group with an observation period of three months. The experiments were conducted under an ethic committee approved protocol in accordance with German federal welfare legislation. One cylinder was implanted into the medullary cavity of each tibia. Postoperatively and weekly, radiographic controls were performed. The animals were clinically examined daily. A µ-Computed tomography and a histologic examination of the right bone-implant compound were accomplished. The left implants were taken out of the bone carefully and examined macroscopically.

Results and Discussion: Clinically in the first ten days the implants were tolerated well in both groups. Two weeks postoperatively rabbits with LACer442 implants showed gas bubbles under the skin. Three weeks postoperatively lameness was observed. After the appearance of one rabbit with profound lameness, one rabbit with a tibial fracture and a spontaneous death of another one with suspected pulmonary embolism possibly caused by gas the LACer442 group was aborted due to unexpected clinical problems and the rabbits were euthanized. In comparison the rabbits in the LAE442 group showed neither gas bubbles nor lameness during the complete observation period of three months. Radiographically, rabbits with LACer442 showed a gas generation, changes in cortical bone structure (two rabbits), periosteal reactions (one rabbit) and structural loss of the implants in contrast to rabbits with LAE442. After euthanasia of the rabbits in the LACer group due to clinical problems and explantation of the tibiae, periosteal bleeding could be seen. In one tibia, the bone under the periosteal bleeding was soft. Only two of five implants could be removed out of the left tibiae as whole cylinders, the others were resolved into pieces which contained lots of corrosion products. In the LAE442 group, after three month no changes in bone or implant surroundings could be seen and all cylindric implants could be removed completely. µ-Computed tomography of the right tibiae confirmed the radiographic and macroscopic observations. It showed a strong implant degradation of the LACer442 cylinders in contrast to the LAE442 ones (Fig. 1).

Fig. 1: LACer442 (A) shows a high corrosion rate after four weeks in comparison to LAE442 (B) after three months with only a small less...
dense margin of the implant (white bar = 1mm).

In contrast to LAE442, no bone adhesion at the implant surface could be seen in LACer442 implants. Histologically, more bone cavities in the cortical bone were found in the LACer442 group. Both groups showed no signs of inflammation. Endosteal bone growth towards the implant was only found in tibiae with LAE442 implants. The osteogenic potential of magnesium alloys, which is described in other studies (3,4,5) was seen in LAE442 but could not be observed for LACer442. Possible reasons could be sudden and profound gas formation and the rapid corrosion process.

Conclusion: This study shows the unexpected result, that minor changes in the composition of the Rare-earth fraction of the magnesium alloys profoundly influence the in-vivo-corrosion rate and biocompatibility. The positive influence of the Rare-earth fraction on corrosion rate and biocompatibility in the magnesium alloy seems to exist only in use with the composition metal. It should be the aim of further studies to find out the reasons for these profound differences in corrosion rate and biocompatibility due to the Rare-earth element cerium as a replacement for the Rare-earth composition metal.

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References:
Metal ions like Pb\(^{2+}\) impair synaptic transmission by interfering with membrane channels and intracellular signaling pathways

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While some metallic species are essential (e.g. zinc or selen), others (e.g. lead or mercury) have solely toxic effects (cytotoxic, neurotoxic, mutagenic, carcinogenic). This review summarizes some major findings in regard to the cellular mechanisms of lead (Pb\(^{2+}\)) neurotoxicity. Recently we have demonstrated that different metal compounds (trimethyltin, arsenic trioxide, cisplatin) interfere with calcium signaling at different levels (Florea et al., 2008, Splettstoesser et al., 2007; Günes et al., 2008, Büsselberg and Florea, 2005). This is also true for the neurotoxicity of lead. With regard to synaptic transmission, there are at least three major target sides for lead (two at the pre-synaptic membrane and one at the post-synaptic):

1.) **Presynaptically:** the arriving action potentials open voltage gated calcium channels, resulting in calcium currents through those channels and consequently in a rise of the intracellular calcium concentration. This is the adequate signal to trigger signal pathways which cause the fusion of the synaptic vesicles with the presynaptic membrane and therefore results in the release of transmitter. Lead impairs calcium entry through those voltage gated calcium channels (predominantly L-type channels; Büsselberg et al., 1994) and therefore reduces the calcium rise and consequently the transmitter release.

2.) While lead acts from the extracellular side to block voltage gated calcium channels it could directly bind to calcium binding sides of calcium dependent proteins within the neuron after entering. As several intracellular pathways are activated by Ca\(^{2+}\), Pb\(^{2+}\) possibly prevents it the function of these signaling cascades. As described before this will result in a reduced amount of neuro-transmitter release in the synaptic cleft.

3.) **Postsynaptically:** transmitters will activate membrane channels which will result in membrane currents and therefore in a change of the membrane potential. Some of these postsynaptic channels (e.g. the NMDA receptor channel complex) conduct Ca\(^{2+}\) ions, which results – similar to the pre-synaptic side - in calcium entry and therefore in a depolarization of the postsynaptic potential.

Since lead binds to the calcium binding site within the channel, the calcium current through the NMDA activated channel is reduced (Uteshev et al., 1996, Büsselberg et al., 1997) and therefore resulting in a reduced postsynaptic depolarization which is less likely to reach the threshold potential to trigger subsequent action potentials.

While lead most likely also impairs other cellular functions (e.g. PKC, ATPases and calcium release and re-uptake into the stores) the described actions occur at low micromolar concentrations and will impair synaptic function overall. Therefore these mechanisms could be a major reason why lead reduces function of the nervous system including memory and learning.

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Effects of respirable particles on lung tissue: Comparison of in vitro and in vivo testing

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Fine and ultrafine particles play an increasing role in materials science as they may reach many parts of the body. Airborne, poorly soluble particles (PSP) particles smaller than 1 µm enter alveolar regions of the lung via inhalation. There, they are taken up by alveolar macrophages (AM) which, depending on the type of particle, react with specific responses. This process is believed to largely contribute to pathologic processes such as inflammation, lung fibrosis or even cancerogenesis. Responses of alveolar macrophages to fine or ultrafine particles can also be studied in vitro. The question was whether or not it is possible to predict effects of PSP on lung tissue from in vitro studies on AM.

AM were lavaged from lungs of healthy donor animas (guinea pigs, rats) and cultured in MEM-medium. In some studies the macrophage cell line NR8383 was used. Various PSPs (different quartz floors, corundum, metal oxides) were suspended in the same medium by brief ultrasonic treatment and pipetted onto AM; in most experiments, a concentration of 15-120 µg particles per 10^6 AM was used. To describe the biological activity of PSP in vitro, we measured lactate dehydrogenase (LDH), glucuronidase, TNF and release of reactive oxygen species (ROS) all released into the supernatant. Vitality and cell damage were also measured. For in vivo studies PSP were intratracheally instilled into the lungs of female Wistar rats (200±20 g) using a multi dose regime (0,15-4,8 mg PSP per lung, 10 animals per group and time point). Follow up times were 3, 21 and 90 days. To determine biological activity in vivo, we examined broncho-alveolar lavage parameters (differential cell count, protein, Fibronectin, TNFα) and analysed cryosections for occurrence of P53, Ki67-antigen and oxidative DNA damage, reflected by 8-oxo-guanine.

Data presented will give an overview of older, partly methodological work mainly focussing on effects of corundum, silica earth and various quartz floors. There will also be a comparison of effects of various metal oxide particles, such as nanostructured TiO2 P25. It will be shown that e.g. various quarz floors with similar chemical composition elicit differential biological effects and can be differentiated from one another if multiple parameters (see above) were compared. Furthermore, results from limited published epidemiologic studies suggest that harmlessness or toxicity of a given material is reflected not only by in vivo studies on rats, but also by in vitro studies on isolated AM. A comparison of in vitro and in vivo results obtained with nanostructured materials will also be demonstrated.

We suggest that the toxicity of PSP can be estimated from in vitro testing results, if comparisons are based on a meaningful combination of parameters. A standardized battery of in vitro tests may thus become part of a tiered approach currently needed to assess the toxicity of PSP.
Session: **Computational Methods for Preoperative Planning**

in presentation order:

A. **Kecskemethy**, Duisburg, Germany  
*Artefact reduction in marker-based gait analysis by integration of MRI data.*

J. **Pauli**, Duisburg, Germany  
*Segmentation of Muscle Sartorius with Tendon Attachment Sites from Magnetic Resonance Images*

T. **Ricken**, Essen, Germany  
*Simulation of biodegradable implants for bone replacement with a multiphase theory.*

M. **Rychlik**, Poznań, Polen  
*Application of PCA and RTG Images for 3D Reconstruction Geometry of the Bones*

T. **Mallepree**, Duisburg, Germany  
*Pre-surgery planning in Orthopedics by means of Medical Rapid Prototyping*
Introduction: Marker-based gait analysis allows accurate and quantifiable documentation of human gait and motion characteristics. For this reason, this method is widely used for the diagnosis of pathological walking patterns and the rating of therapeutic measures. A drawback of existing systems are artefacts in the motion data caused by skin motion as well as the special knowledge necessary for the processing and interpretation of the results.

This paper describes a novel simulation environment for marker-based gait analysis which combines the data gathered in gait-lab measurements with MRI recordings in order to reduce the artefacts of the gait-lab measurements. The model generated from the combined data simulates the individual musculoskeletal system of a patient and provides full motion simulation in a 3D environment. This patient-specific mechanical model can be employed for direct medical interpretation by using methods of kinematics, dynamics, segmentation and optimization. Therefore the new gait analysis software can be used to produce clinically employable results in hospitals, surgeries and individual practice without the need of additional technical staff.

Materials and Methods: The new simulation system combines gait analysis data with MRI measurements into a patient-specific biomechanical model. To this end, bone shapes are reconstructed from medical image data using a segmentation algorithm and functional axes are identified by fitting of super-quadrics [2],[3]. The matching of the segmented bones with the gait lab data and the motion simulation is performed with the multi-body library Mobile-C++, which has already been successfully applied for the development of industrial virtual design environments e.g. for robots, satellites, vehicles and complex industrial equipment.

Using the mechanical model as a dynamical kernel, the system provides full motion simulation in 3D environment. In contrast to conventional raw data rendering techniques, which are difficult to interpret for practicing doctors, the simulation system offers an intuitive interface which is adapted to clinical requirements. The pre- and post-processing includes evaluation of raw data input according to medical criteria, comfortable user interface, as well as interactive three-dimensional visualization with head mounted display or projection techniques.

Results and Discussion: The described kinematical model allows one to build up the motion of the legs by starting at the feet and proceeding to the hip joints, where a kinematic closure condition is formulated. In this way, more exact bony motion reconstruction is possible, as shown in respective simulations and validation runs. In standard gait analysis, the model avoids huge, unrealistic internal/external rotations of the tibia and the femur, without requiring more markers than when using the .Plug-In-Gait. model.
References:

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NOTES:
Introduction: Only few publications are devoted to the extraction and partitioning of soft tissues from medical images, like muscles [1] and ligaments [2]. We present a method for the segmentation of muscle sartorius with tendon attachment sites (TAS) from 3D Magnetic Resonance Tomography images. The segmented muscle, including the positions of the extracted tendon attachment sites, can be used for mechanical force analysis in orthopedic surgery planning.

Materials and Methods: Two kinds of statistical shape (STS) models are created automatically from a collection of sample images [3]. The first kind of models comprises two 2D contours of ones which include the TAS of the muscle sartorius. Each 2D bone STS model is used to search for the image that includes the TAS of the muscle sartorius in the set of MR images. By using the searched images, the vertical length of the muscle sartorius can be calculated. The second kind of model is used for extracting the 3D muscle sartorius (Fig. 1). It is adjusted for muscle segmentation via a model-based active contour method (Fig. 2).

Results: Comparing the segmented muscle and the corresponding TAS positions of our automatic method with manual segmentation by an expert, the accuracy of our method is quite acceptable (Fig. 3).

Discussion: A new method is proposed for segmentation of muscle sartorius and extraction of TAS positions. The method needs scarce user interactions and will be further improved for practicable clinical use.

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3. T. Cootes, C. Taylor, D. Cooper, et al., Active Shape Models - Their Training and
NOTES:
Simulation of biodegradable implants for bone replacement with a multiphase theory

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Biodegradable implants temporarily support the body during the regeneration and degenerate at the same rate as the bone is built up again. Bone tissue grows into the porous structure of the implant and fills out the space which is left by the degrading implant. Such materials have been developed and successfully tested in vitro and in vivo, see [1]. A complete understanding of both the mechanical properties and the degradation behavior, is necessary for optimal application in the clinical practice. In order to achieve this aim we developed a numerical calculation concept to simulate the mechanical behavior of the porous implant coupled with the complex biodegeneration behavior. The macro-mechanical Theory of Porous Media (TPM) provides a continuum-mechanical and volume-averaging description of the multi-phase implant, see Fig 1 and [2]. In addition to the solid and fluid/gas phase, we consider a chemical active solution, which is responsible mainly for the speed of implant biodegeneration and it is therefore essentially necessary to consider it in the model. This description leads to a strongly coupled set of differential equations which allows for the determination of the four unknown quantities: motion of the solid matrix $u_s$, fluid pressure $\lambda$, volume fraction of solid matrix $n_s$ and amount of concentration $n_N$.

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Fig. 1: Macro structure, micro resolution and model idealization.
Introduction. 3D CAD models and other techniques knowing from mechanical engineering – like reverse engineering, rapid prototyping, FEM analysis, virtual reality – are widely used in biomedical areas. The 3D virtual models have numerous applications such as visualization, medical diagnostics (virtual endoscopes), pre-surgical planning (simulations of surgical operations) and others. The acquisition and processing of three-dimensional models with complicated shapes (biomedical objects) becomes the important issue for further applications.

In this paper author present non-invasive method of the 3D reconstruction geometry (CAD models) of the bones, basis on RTG Images and knowledge form PCA (Principal Component Analysis) database.

Methods. For computation of database the Principal Component Analysis (PCA) and 3D scanning methods was used. PCA analysis [1, 2] (low dimensional decomposition) gives information about three dimensional geometrical features of biological objects set (human bones). The shape of the every object is represented in the database as the 3D grid. Each grid is described by vector:

\[ S_i = [s_{i1}, s_{i2}, \ldots, s_{iN}]^T, \quad i = 1,2,\ldots,M, \]

where \( s_{ij} = (x, y, z) \) describes coordinates of the nodes (FEM grid) in Cartesian coordinates system, \( M \) is the number of the objects which are in database, \( N \) is the number of the FEM nodes of single object. The differences between mean and object that is in database are described by the deformation vector \( \bar{S}_i = S_i - \bar{S} \). The statistical analysis of the deformation vectors gives us the information about the empirical modes. Modes represent the features: geometrical (shape), physical (density) and others like displacement and rotation of the object. Only few first modes carry most information, therefore each original object \( S_i \) is reconstructed by using some \( K \) principal components:

\[ S_i = \bar{S} + \sum_{k=1}^{K} a_{ik} \Psi_k, \quad i = 1,2,\ldots,M, \]

where \( \Psi_k \) is an eigenvector representing the orthogonal mode (the feature computed from data base), \( a_{ik} \) is coefficient of eigenvector.

Results and Discussion: Algorithm of the method is following (fig. 1.). Searched three-dimensional object are represented by the set of RTG images (minimum two images from different directions). These RTG images are compared with DRR - Digitally Reconstructed Radiographs [3] from database. Data base includes DRR images and set of the modes and coefficients. (received from PCA). After comparison of images, the most similar objects from database are selected. In the next step we manipulate the coefficients of modes as long as the minimization of the mean square deviation of the images RTG and DRR is accomplished. Finally we receive reconstructed 3D model in CAD system.

![Fig. 1: Algorithm of the method and result of PCA for vertebra bone (empirical database)](image)
completely new values (there is no similar shape in data base). In the next step we compare the DRR images of the searched and created vertebra (fig. 2) and manipulate of the coefficients of modes. To compute the values of coefficients for all modes Jacobi criterion was used. The final result of this experiment is the solid CAD model (fig. 2).

![Fig. 2: Correlated images (from left): RTG image of searched object, DRR image of created (deformed) model, image of CAD solid model searched vertebra (left side) & reconstructed vertebra (right side)](image)

PCA analysis was also used for extraction of mean shape and features of real femur bones database obtained in 3D scanning process. The mean shape and features of real bones were presented and discussed. Presented method makes possible reconstruction of three-dimensional shape of the complex geometry in CAD system, basing on the few RTG images and knowledge about object geometry recorded in database. This method use full volume information from RTG images and they can be used for reconstruction of the biological and non-biological objects (e.g. mechanical objects). Application of three dimensional Principal Component Analysis make possible extraction of mean shape and geometrical features (which describes principal deformations in analyzed group of objects) of biological objects set. This method can be used to creation of full three dimensional anthropometric database. Advantages of three dimensional anthropometric database is possibility of measured any dimension on the surface of the bone (3D surface of the mean shape) without new research and measurements processes on set of bones.

References:

Pre-surgery planning using Medical Rapid Prototyping

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The planning of complex surgical procedures necessitates reliable models of the individual human anatomy. With a physical model at hand, it is possible to rehearse different surgical plans realistically. Medical modeling techniques known as biomodeling provide the possibility to reconstruct three-dimensional (3D) models of anatomical templates of parts of the human body. Recent studies introduced Rapid Prototyping (RP) applications to specific research areas of surgical planning and manufacturing of personalized models.

Planning a hip joint implantation, an understanding of the anatomy of the hip is essential. Typically, two-dimensional tomographic images are used generated by magnetic resonance imaging (MRI) or computed tomography (CT) scanners. The objective of the present study was to establish the methods and parameters necessary in order to provide high accurate 3D models of the hip rapidly. Anatomical data derived from scanned medical images. Data sets were segmented and 3D reconstructed. After an evaluation of necessary parameters, the reconstructed models were converted to the stereolithography (STL) format. Finally, the quasi-generative RP application of CNC milling is selected for fabrication. Appropriate machining parameters were determined.

The main finding of the study was the good agreement observed between different data sets from which RP models of the hip could be derived by evaluated parameters. The model accuracy was mainly related to the original tomographic scan. Scanning the hip for biomodeling, CT showed optimal suitability for segmentation. The 3D reconstruction result was suitable if an optimum adjustment of the meshing parameters of triangle reduction and smoothing was chosen. The quasi-generative RP application of CNC milling showed its suitability for generating accurate models rapidly.

Avoiding inaccurate 3D models, the evaluated parameters will lead to high-accurate and process feasible RP models even of anatomical models as the hip. Gaining insight in the interplay of parameters necessary for modeling complex anatomies enables to provide accurate models in a decreased processing time. This will provide new possibilities in optimizing complex surgical procedures.
Session: *Composites with BMP-2*

in presentation order:

K. **Koczur**, Aachen, Germany  
*Manufacturing of novel materials for BMP coupling process*

A. **Wutzl**, Vienna, Austria  
*Bone morphogenetic proteins 2, 5 and 6 in combination stimulate osteoblasts but not osteoclasts in vitro*

H. **Jennissen**, Essen, Germany  
*BMP at the Solid-Liquid Interface*

K. **Zurlinden**, Essen, Germany  
*BMP-2 Loaded Microstructured Phycogenic Bone Substitutes*

R. **Ewers**, Vienna, Austria  
*Biocoating of Hydroxy-apatite of Phycogenic Origin: In Vivo and Clinical Results*

T. **Sänger**, Essen, Germany  
*Accelerated Hydrolysis of Foamed rhBMP2/Poly-(D,L)-Lactide*
The resorption process of biodegradable implants inside the human body is limited and strongly depends on the size of component. It is known that bone growth can be supported by so-called Bone Morphogenetic Proteins (BMP). The protein-coupling mechanism and desorption kinetics of the BMP on mineral surfaces, however, is not completely understood.

The presented study is part of a cooperative project that is focused on the characterization of BMP coupling to mineral surfaces and the explanation of the in vivo behaviour of implants coated with BMP proteins. The major objective of the first part of this project is to develop an innovative, novel material which fulfills several requirements. The first condition of the material for the BMP-coupling process is the specific surface, which should be functionalized in a way that the BMPs can be easily coupled to the surface by active hydroxy groups. Several materials (composite of bioactive glass and β-tricalcium phosphate phases, composite of the hydroxyapatite and β-tricalcium phosphate phases, pure wollastonite, Algisorb, and Algipore) were used to manufacture compressed cylindrical specimens, and were analyzed with respect to their properties. At first, each of those materials was characterized, using scanning electron microscopy (SEM), dispersive X-ray spectroscopy, X-ray diffractometry, and X-ray fluorescence analysis. The second part of the experiments was focused on analyzing the ability of those materials to populate the BMP on their surface.

First results have shown that on the surface of the composite of bioactive glass and β-tricalcium phosphate (which after thermal treatment at 1000°C for 5 hours changed the phases into rhenanite and wollastonite) the coupling of BMPs is possible after pretreatment with the different acids. Moreover, both phases - rhenanite and wollastonite - are known as bioactive and even biodegradable materials. Very good properties present also the composite of the hydroxyapatite and β-tricalcium phosphate phases, because the hydroxyapatite phase exhibits the presence of the OH- groups on the surface and the proteins can be easily coupled without a surface activation process (e.g., acid treatment), whereas the β-tricalcium phosphate exhibit very good bioresorbable properties.
NOTES:
Bone morphogenetic proteins 2, 5 and 6 in combination stimulate osteoblasts but not osteoclasts in vitro.

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Objective: Bone regeneration is required for fracture healing, and different procedures have been used to promote osteogenesis requiring multiple BMPs. In this study, we assessed the effects of BMP-2, BMP-5 and BMP-6 alone and in combination on osteoblast and osteoclast generation.

Material and methods:
To compare osteoclastic potency of each BMP, osteoclasts of primary murine bone marrow cells were treated with BMP-2, BMP-5 and BMP-6. Subsequently, cells were stained for tartrate-resistant acid phosphatase (TRAP). The same combination of BMPs were used to assess their potential to enhance osteoblasts using a mineralisation assay and real time PCR analysis of collagen type-1, runx2 and osterix as well as RANKL and OPG.

Results: The presented data show that the combination of BMP-2, BMP-5 and BMP-6 did not enhance osteoclastogenesis compared to the single use of either BMP. However, the effects of BMP-2, BMP-5 and BMP-6 in combination on osteoblasts were additive for mineralization and osterix.

Conclusions: This study demonstrates that the combination of BMP-2, BMP-5 and BMP-6 stimulates osteoblasts while it reduces osteoclastogenesis. Thus, the synergistic use of various BMPs might improve efficacious bone regeneration in a clinical setting.
Engineering BMP-2 at the Solid-Liquid Interface.

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The morphogen BMP-2 is a water soluble protein for which the type 1 and type 2 receptors constitute the only known specific and stoichiometric binding-sites. Thus for clinical applications involving carrier systems it is pertinent to study the adsorption and desorption of such morphogens at the solid-liquid carrier interface. For the immobilization of bioactive proteins on biomaterials two major types of artificial bioactive surfaces have been suggested: (i) chemotactic and (ii) juxtacrine. Here, only chemotactic release surfaces will be treated. In the absence of a physiological carrier, materials have to be screened, in order to find a suitable delivery system. According to present knowledge a carrier with immobilized BMP should fulfill at least three criteria with the following parameters: (i) high capacity (above biological threshold; mg BMP/g; µg/cm²), (ii) long release time (half-life, days/months) and (iii) high biological activity of released BMP (in vitro test). We have therefore studied a number of biomaterials based on the above regimen, such as: collagen, hydroxy apatites (HAP) (e.g. BioOss, Geistlich, NuOss, ACE Surgical Supply Co.) and blends of HAP and tricalcium phosphate (TCP) (e.g. Aligipore, Algisorb, Algoss GmbH or Bonit, DOT GmbH). In addition we tested bioglass, polylactides (PDLLA) and metals such as titanium, cobalt chromium alloys and medicinal steel. For these materials data according to the above criteria have been obtained. From the experiments it can be concluded that the single most important parameter is the binding constant (Kₐ) involved for BMP as a result of the binding procedure to the surface. This constant is crucial for the biological activity and determines the half-life of release, which in the case of a closed system is decisive for the tissue-equilibrium concentration obtainable for effective bone induction. This concentration should be in the physiological range (10⁻⁸⁻¹₀⁻⁹ M) to exclude adverse effects. The present experiments indicate that binding constants (Kₐ) for implant-immobilized rhBMP-2 in the range of 10¹⁰⁻¹₀¹² M⁻¹ are realistic for our biologically active systems.
NOTES:
Hydrophobic and Hydrophilic Adsorption of rhBMP-2 on Phycogenic Bone Substitutes

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In maxillofacial/orthopedic surgery application of autologous bone graft for defect filling due to trauma or cancer is a common procedure. However due to the limited availability of autologous bone and due to the donor-site pain and morbidity the use of inorganic bone graft substitutes becomes widely accepted as these materials show good biocompatibility and osteoconductive properties [1]. In order to allow healing of larger defects and accelerate the healing of smaller defects enhanced bone growth has to be achieved. This is feasible by coating these materials with bone morphogenetic proteins like BMP-2. At present collagen sponges are clinically used as carrier for BMPs (InductOs, Ossigraft). However it has been shown that these materials bind only minor amounts of BMP-2 [2] with half-lives in a range of 2-3 days [3].

We show here that rhBMP-2 is immobilized in bioactive form on HA/TCP surfaces (Algisorb, Algipore, Algoss GmbH) in an amount of up to 7 mg/g by two methods. The protein can either immobilized in a hydrophilic manner after boiling the material in water or in a hydrophobic manner after chemical modification of the surface with APS (3-aminopropyltriethoxysilane) [4].

The description of the amount of bound protein as a function of the equilibration concentration of rhBMP-2 shows biological activity comparable to the positive control (soluble BMP-2).

![Figure 1: Immobilization of 125I-rhBMP-2 on modified and unmodified Algisorb.](image)

A: Hydrophobic Adsorption of rhBMP-2 on APS-modified Algisorb
B: Hydrophilic Adsorption of rhBMP-2 on unmodified Algisorb

The amount of bound protein is shown as a function of the equilibration concentration of rhBMP-2.

### References:

The use of autogenous bone has long been considered the “Gold Standard” for augmentation procedures. A number of papers have shown that synthetic materials have now been developed which produce comparable results in new bone formation and remodeling. This lecture will look at and compare the materials that are available. The new frontiers of bone regeneration brings materials which have natural porous scaffolding, cell chambers absorptive capacity and ideal characteristics for enhanced bone production. The material we prefer is a natural product synthetically manufactured out of marine algae.

Discussion will also offer ideas on various ways to enhance bone regeneration and to bioengineer resorbable materials by biocoating these materials. New developments utilizing bio-tissue engineering, bone morphogenic proteins, bone inductive hormones and synthetic peptides will be discussed. Referring to these excellent results in animal experiments I will show what are the new developments concerning biocoating materials with BMP’s. I will show which inductive materials we are already using in patients and what will be the future.
Release of rhBMP-2 under Conditions of Accelerated Hydrolysis of Foamed rhBMP-2/Poly-(D,L)-Lactide

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Introduction: We showed that it is possible to incorporate large amounts of biologically active recombinant human bone morphogenic protein 2 (rhBMP-2), up to 3.4 mg/g PDLLA into a PDLLA-matrix [1] by foaming PDLLA-powder with supercritical CO2 at 100 bar and 45°C [2]. In the present work, foamed rhBMP-2/PDLLA tablets (0.7 mg rhBMP-2/g PDLLA) were analyzed, concerning accelerated hydrolysis rate and release of incorporated rhBMP-2. To accelerate the hydrolysis rate of rhBMP-2/PDLLA tablets, the reaction rate was increased ca. 7-fold [3], by incubating the tablets directly below the glass transition temperature of PDLLA at 50°C in PBS-buffer (pH 7.4).

Materials and Methods: RhBMP-2 was prepared in E.coli and labeled with 125I according to the Chloramine-T method [4]. PDLLA (Resomer® R207) was purchased from Boehringer-Ingelheim. RhBMP-2/PDLLA-tablets (34 ± 5 mg, 0.70 mg rhBMP-2/g PDLLA, 125I-rhBMP-2 with specific radioactivity of 5153 Cpm/µg) were made as described [1]. PDLLA-powder was sterilised by γ-irradiation at 24 kGy.

The hydrolysis of PDLLA was accelerated by incubation at 50°C [3]. To determine the change in the polystyrene equivalent molecular weight (PSE-Mw) [5] of PDLLA polymer chains, by size exclusion chromatography (SEC), the tablets were freeze dried and dissolved in chloroform. pH-measurement of the incubation buffer was made at room temperature. Tablets, loaded with 125I-rhBMP-2, were incubated for different time spans (one hour up to 40 days) in borosilicate vials with PBS-buffer (5.4 ml, pH 7.4) at 50°C without buffer change, so that the released rhBMP-2 accumulated in the incubation buffer (soluble rhBMP-2). At the indicated times, the incubation buffer was pipetted out of the vial and the released 125I-rhBMP-2 as well as the polystyrene equivalent molecular weight (PSE-Mw) from PDLLA polymer chains during accelerated hydrolysis:

125I-rhBMP-2 were released. Followed by a slow phase, between day 3-19, where after 19 days, 12.8 ± 2.3 % (residual 81.7 ± 4.1 %) 125I-rhBMP-2 were released. Between day 19-40 the tablet began to disintegrate and a further release phase occurred, where after 40 days, 25 ± 3.8 % (residual 54.4 ± 5.6 %) of 125I-rhBMP-2 were released. From day 19 - 40 the pH-value decreased from 7.4 to 3.

Fig. 1: Release of 125I-rhBMP-2 from foamed PDLLA tablets and pH-development under conditions of accelerated hydrolysis

PDLLA powder was γ-irradiated at 24 kGy. Tablets were loaded with 0.70 ± 0.06 mg rhBMP-2/g PDLLA (=100 % with 122245 ± 17783 Cpm) and n=3 tablets were incubated in PBS-buffer (5.4 ml, pH 7.4) at 50°C without buffer change for indicated time spans.

Decrease of polystyrene equivalent molecular weight (PSE-Mw) from PDLLA polymer chains during accelerated hydrolysis:

γ-sterilisation, as stated in methods, at 24 kGy leads to a decrease of PSE-Mw of the PDLLA polymer chains from 200000 g/mol to 103340 g/mol. In Fig.2 it is shown, that incubation after γ-sterilisation in PBS-buffer (pH 7.4) at 50°C resulted in a rapid decrease of the PSE-Mw, from 103340 g/mol to 9340 g/mol within day 1-19. After 19 days the PSE-Mw was too small to allow detection by size exclusion chromatography.
Fig. 2: Decrease of PSE-Mw of PDLLA polymer chains during accelerated conditions
PDLLA tablets (n=3) with an initial PSE-Mw of 103340 g/mol were incubated in PBS-buffer (5.4 ml, pH 7.4) at 50 °C without buffer change for different time spans. At indicated times, the tablets or residual PDLLA were taken out of the buffer, freeze dried, dissolved in chloroform and the change of the PSE-Mw from PDLLA polymer chains was determined with SEC.

Conclusions:
I. Incubation of foamed rhBMP-2/PDLLA at 50 °C results in an accelerated hydrolysis of the PDLLA.
II. The hydrolysis of PDLLA (t1/2 ~ 6-7 days) is much more rapid than the release of soluble rhBMP-2 (estimated t1/2 >> 40 days).
III. Insoluble rhBMP-2 is possibly retained in PDLLA with low PSE-Mw (< 9000 g/mol).

References:
Session: **Hard Ceramic Couples in Orthopedics**

in presentation order:

M. **Morlock**, Hamburg, Germany  
*The Tribology of Squeaky Hip Joints*

R. **Pourzal**, Duisburg, Germany  
*Wear Mechanisms on a Squeaky Alumina-on-Alumina Hip Prosthesis -A Case Report-

T. **Pandorf**, Plochingen, Germany  
*Alumina matrix composites for Arthroplasty*
Introduction: Charnley observed as early as 1979 that the presence of certain conditions could lead to squeaking in hip replacement patients. The squeaking itself is seldom permanent and tends to disappear after a few days. Owing to the tendency of the phenomenon to come and go, there is a general lack of sound clinical data on the occurrence of squeaking [KKWS08, MNJS01]. Researchers have still not been able to clearly identify the causes of squeaking or specify the primary contributing factors. However, it can be assumed that certain causal relationships exist between the occurrence of squeaking, the design of the artificial hip, its positioning and the specific loading situation.

Fig. 1: Hip simulator for acoustic measurements.

Fig. 2: Time-frequency representation of a squeaking sound: A squeaking sound arises and ceases (amplitude axis) in the course of the movement cycle (time axis).

Fig. 3: Sample calculation result: Maximum deformation of a press-fit acetabular cup during squeaking. The cup moves in a certain relation to the ball head which gives rise to a coherent vibration perceived as a squeaking sound.

Fig. 4: Chains of action & investigation methods: In addition to structural dynamics and acoustics, tribological factors and ossification also influence noise development.

The purpose of this project is to identify the causes of squeaking and the main factors that contribute to its development, focusing on friction-induced vibrations. In numerous technical systems, the vibrations that cause audible squeaking, arise in connection with sliding motions or in connection with the transition from a sticking to a sliding state. Typical examples include the sound generated...
by stringed instruments, the squeaking of friction brakes and even the rumbling made by an avalanche. All squeaking is based on physical mechanisms that generate a certain degree of vibration beyond the desired smooth and even sliding motion [Hof06, AA06, Hof07]. The so-called self-generating vibrations produced in this manner then lead to an acoustic epiphenomenon that we perceive as squeaking.

**Experimental Measurements:** To arrive at an initial characterization of the phenomenon, airborne-sound measurements and vibration measurements were taken in a special hip simulator (Fig. 1). Mechanical testing, clinical data and anecdotal reports indicate that squeaking is only generated in connection with specific movement and load configurations. The squeaking itself is manifested in a sustained perceivable sound that is dominated by a certain frequency (Fig. 2). This allows to clearly demarcate the phenomenon of squeaking from other phenomena such as clicking sounds. The frequency associated with squeaking is directly related to the stiffness of the bodies involved. Significant differences (e.g. relating to cup stiffness) have already been identified.

**Modelling and Simulation:** The physical mechanisms at work on the sliding surface itself are difficult to examine experimentally. A computer-assisted computing model was developed in order to gain a better insight into these mechanisms, and in order to be able to vary the contributing factors independently of the experimental conditions. This allows to examine joint components associated with specific movement and load configurations for the development or absence of squeaking (Fig. 3). The initial results show that an increased risk of squeaking exists if individual components have eigenfrequencies close to the excitation frequencies. The components do not need to be in direct contact at the sliding surface. Furthermore, increased friction is required in order to achieve vibration amplitudes which result in audible noise.

**Summary and Outlook:** While the experimental and simulation supported research that has been conducted so far indicates that the occurrence of squeaking in artificial hips is a multifactorial phenomenon, numerous relationships have not yet been sufficiently explained and are in need of further investigation. Given that squeaking only occurs in certain very specific movement and load parameters, a step-by-step approach to delimiting the critical parameter areas of the basic chains of action is being taken so as to enable an effective means of intervention for designers and clinicians (Fig. 4). The research is being conducted in the context of a global project involving various renowned laboratories.

**Acknowledgement:** This study has been supported financially by CeramTec AG.

**Reference:**

Wear Mechanisms on a Squeaky Alumina-on-Alumina Hip Prosthesis
-A Case Report-

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Introduction: Lately, there have been anecdotal reports of squeaking in ceramic-on-ceramic (COC) joints. Although reported as uncommon in a recent study [1], squeaking of COC joints warrants investigation as it may signal abnormal wear of the bearing surfaces. In this study, an alumina-on-alumina hip was examined that was explanted due to persistent squeaking. It was hypothesized that the squeaking was associated with degradation of the bearing surfaces. We also sought to investigate the cause of any such degradation.

Material and Methods: A Trident ceramic hip (Stryker Corp., Kalamazoo, MI) with a 36 mm diameter head was revised after 32 months because the patient experienced severe audible squeaking in his hip during motion. In this implant, an alumina ball articulates against a titanium alloy-backed alumina cup insert. The alumina is Biolox Forte (CeramTec AG, Plochingen, Germany). The cup inclination angle measured from a frontal plane radiograph was 68°. The surfaces were examined by means of scanning electron microscopy (SEM) using a Leo Gemini 1530 (Oberkochen, Germany) with the application of electron dispersive x-ray spectroscopy (EDS) and by transmission electron microscopy (TEM) using an Philips EM400 (Eindhoven, Netherlands).

Results: Immediately visible on the head are three dark streaks, the largest being 14 mm x 1 mm (Fig. 1), with one end close to the pole. Examination by SEM-EDS revealed the streaks to be smeared titanium-containing material, consistent with evidence of impingement found on the inner side of the overhanging edge of the titanium alloy backing. An EDS analysis of the impinged areas revealed severe material transfer of alumina. On closer visual examination, the heads surface was separated at the pole into a polished and a high roughened area. The roughened area is highlighted with a green tint in Fig. 1. It is of interest that the largest metal streak spans almost exactly the roughened area on the head. The surface exhibited an average roughness Ra in the dulled area of 133 nm versus 3.8 nm Ra in a nearby still polished area. This considerable difference was reflected in the appearance of these surfaces under SEM examination. The grain structure was clearly visible for both surfaces, but grain pullout and fracture were far more extensive in the roughened area (Fig. 2).

Fig.1: Head insert. The roughened area is highlighted with a green tint. The dark streaks on the head are smeared metal.

The bulk material exhibited constantly the presence of hexagonal rhombometric lattice (c/a=2.73) which is a stable phase. The TEM analysis of the sub-surface zone of the polished area revealed the presence of different phases. Besides the stable hexagonal rhombometric phase two hexagonal primitive lattices occurred. One has a lower c/a ratio of 1.63 and the other a higher of 4.02. Both phases are meta stable. The diffraction pattern analysis of the roughened area revealed no occurrence of phase transformation.

Discussion: Previous studies reported the occurrence of two main wear areas on the articulating surface of the head [2]. One in the normal articulating area called low wear area, and one close to the head’s rim called stripe wear area. Surprisingly, in our case, there was no evidence of stripe wear or of head impact against the edge of the ceramic insert. Additionally, the observed wear features were unrelated to previously reported micro-separation of COC joints. In one part of the low wear area the pull-out effect led to a higher density of grooves than previously observed in other studies. Grains and grain fragments of µm-size were released into the system and were expected to cause third-body abrasive wear. However, almost no scratches of µm scale occurred anywhere on the articulating surface. The brittle particles were possibly milled down to particles of nm size causing surface fatigue leading to a highly polished area surrounding the roughened area.
The steep inclination angle (68°) of the cup led to some degree of edge loading against the head, which increased the contact stresses and consequently the wear rate. In a recent study [1], cup orientation and edge loading were reported as factors contributing to squeaking in COC hips. The steep inclination angle also appears to have led to subluxation of the head, leading to contact with the overhanging edge of the metal backing, and smearing of metal on the articular surface.

**Fig. 2:** Scanning electron micrographs of the heads articular surface in the polished area (left) and the roughened area (right).

The position of the large metal smear across the wear band on the head (Fig. 1), suggests the smear is directly connected with the abnormal wear pattern. It is speculated that the metal smear reduced the fluid lubrication of the joint during its flexion-extension sweeps, leading to grain fracture and surface roughening. The higher roughness will itself disrupt fluid film lubrication by decreasing the lambda ratio, making surface roughening a progressive process. The squeaking sound may then result from stick-slip of the roughened surfaces against each other under load. It may be concluded that the squeaking sound made by the joint was associated with degradation of the bearing surfaces.

The results of the sub-surface phase analysis have shown the occurrence of a phase transformation in the polished area, where pure sliding motion was dominant. In the roughened area no phase transformation was observed. In this area phase transformation is dominated by whole grain pull-out and fracture.

The hypothesis that squeaking in COC joints may provide an early warning signal of joint degradation should be further investigated.

**References:**

2. Nevelos, J.E., Analysis of retrieved alumina ceramic components from Mittelmeier total hip prtheses, Biomaterials 20 (1999), 1833-1840
Introduction: Over the past 30 years, applications for ceramics in orthopaedics have continuously increased. The first use of ceramics in this field was a pure medical-grade alumina starting in 1971. More than 5 million components have been implanted since then. Improvements regarding the performance and the reliability of the material have been reached by reducing the grain size in the ceramics and introducing multiple process improvements like proof testing of all components, iso-static hipping, laser etching, and others. Nowadays, the requirements for an artificial hip are becoming more and more challenging. The majority of the patients still want to be active and live a sportive life style. Thus, an increased range of motion together with the request to provide a mechanically safe product demands more safety from the material and new implant designs, leading in most cases to a lower wall thickness.

Material: The resulting requirements for the ceramic components have been met with a zirconia platelet toughened alumina (ZPTA). With its zirconia content of 17 Vol%, this material has an internal reinforcement mechanism while maintaining the hardness due to added chromium. This yields enhanced mechanical strength and fracture toughness with respect to pure alumina. Compared to pure zirconia or alumina toughened zirconia, the risk of instable zirconia transformation and resulting worsened wear behaviour is avoided.

Discussion: The ZPTA has a predominant and unique toughening mechanism: small (less than 300 nm) particles of zirconia in tetragonal state are evenly dispersed in the alumina matrix (av. grain size 600 nm). In the presence of a small defect or crack, the transformation of the zirconia particles in monoclinic state involves a volume increase leading to pressure stresses in the neighborhood of the crack tip reducing the tensile stresses acting on the crack tip leading to increased mechanical strength and fracture toughness. Hydrothermal aging of the zirconia content is extensively discussed. Due to the particular distribution and stabilization of the zirconia particles aging effects are controlled in this material. After very long time of accelerated aging conditions an increase of monoclinic phase is found – however, it is shown that the dynamic and static properties of this ZPTA are not influenced by this effect.

Conclusion: The outstanding properties of the ZPTA support advantageous properties of the final product, e.g. ceramic hard-hard bearings for hip arthroplasty. The burst load of both the ball heads and the inserts are significantly increased. Wear properties at severe conditions, e.g. micro separation are also strongly improved in comparison to pure alumina. Additionally, the increased strength allows for smaller wall thicknesses of the inserts.
Session: **Particles in Hard Metal-on-Metal Bearings**

in presentation order:

**A. Fischer**, Duisburg, Germany  
*Microstructural Alterations of CoCrMo-Alloys under Articulation. Experiment and Computer Simulation.*

**M. Spinelli**, Bologna, Italy  
*Microstructure and Surface Characterization of Metal-on-Metal Hip Joints*

**I. Catelas**, Ottawa, Canada  
*Characterization of Particles and their effect on Cells*

**J. Medley**, Waterloo, Canada  
*MoM Tribology - Reason for Particles*

**P. Beaule**, Ottawa, Canada  
*MoM - The clinical point of view*
Microstructural Alterations of CoCrMo-Alloys under Articulation. Experiment and Computer Simulation.

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Underneath contact surfaces under sliding wear distinct microstructural changes have been found in biomedical materials. These are attributed to the nature of the contact stress (and strain) field as it can be derived from computer simulations on the basis of measured frictional and normal forces. These depict that the von-Mises stresses might have several maxima characterized by differing multiaxialities. According to the nature of these contact stress fields the reaction of materials differs distinctly as well.

The investigation of subsurface areas of retrieved metal-on-metal hip joints and laboratory specimens revealed, that the worn surfaces of these fcc materials in general consist of a nanocrystalline (nc) layer with a thickness of up to 200 nm. This layer is chemically modified by mechanical-mixing, which introduces the interfacial medium into the subsurface volume. These experimental findings have been supported by molecular dynamics simulations.

Below 200 nm one often finds a nc-layer of the same grain size, which has the chemical composition of the base material. Now the interface between the mechanically mixed nc-layer and the underlying one cannot be distinguished by grain size, but only by chemical composition. Thus some of the nanocrystals are not generated by mechanical-mixing but by in-situ recrystallization. In comparison to shear fatigue tests under similar cyclic loads these grains depict the same lattice defects as in a sliding wear stress field. Analytical contact mechanical computer simulations revealed that in these areas a cyclic stress field prevails which again is in good coincidence with the experimental findings.

This contribution shows that computer simulation and experimental validation can support each other in order to understand the complicated interaction of wear mechanisms under sliding wear. But there is no general description available and any aspect requires its own simulation method.
Microstructure and Surface Characterization of Metal-on-Metal Hip Joints Introduction

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Total hip replacement (THR) still registers huge longterm failures (major than 15 yrs). Concurrent mechanical and biological processes leads to implant failure and to revision surgery [1-3]. A way to reduce wear is to create a bearing couple with two distinctly different surface and microstructure properties. Surface modifications are only several angstroms thick and may not last for the life of the implant [4]. If these differences are carefully selected, the resulting couple acts will minimize the effect on its bearing partner. Surface characterization will help in the understanding of the wear mechanisms of MOM hip implants and address alloy selection. With this in mind, this study was aimed at examining carbide morphology and behaviour, as well as matrix wear mechanisms of different Metal-Metal (MOM) wear tested components.

Materials and Methods: Nine metal-on-metal bearing specimens of different size (28-mm, 32-mm, 36-mm) were tested, using a hip joint simulator (Shore Western, USA) under bovine calf serum as lubricant (25v/v balanced with deionised water). The test lasted two million cycles. Metallographic microstructure examination was conducted on the components. The heads were cut perpendicular to the surface [5], the pieces of metallic heads were embedded in an acrylic resin (AcryFix, Struers, Denmark), and then manually grinded with SiC paper from rough to finer paper (P100 to P2000). Polishing was then conducted with 3 microns diamond spray with lubricant on a rotating disk. Finally, the metallic specimens were etched for a few seconds in a solution of 42.5ml 36% HCL and 2.5ml of 35% H2O2. The prepared specimens were then examined both with optical metallographic microscope (OMM – Rechert-Jung MeF3, Austria) and with electron scanning microscope (SEM - Zeiss Evo50) operating at 25kV and equipped with the Energy Dispersed Spectroscopy probe (EDS - Inca Energy-350 Oxford Instruments).

Results: Visual examination of heads and cups surfaces showed no major macroscopic damages. The loaded zone of each configuration exhibits holes and micropits due to carbides loss with different features for the three sizes. The holes were regular in shape with undamaged surrounding material for the 36-mm and 32-mm size (Fig 1a) while they appeared uneven with some apparent smearing effects on the surrounding area for the 28-mm configuration. The SEM analysis showed many possible carbides chains.
components both in grain dimension and in carbides quantity. The higher carbon content of the material improves wear behaviour of the device; in fact, carbides inclusions in the bulk matrix give additional mechanical resistance, in particular to shear forces. SEM analysis put in evidence carbides loss in correspondence of carbides housing with the possibility of leading to third body wear, particularly evident on the small size configuration. Some wear and corrosion phenomena need long time before manifestation so that the tested specimens showed only the initial phase of such processes. Longer test duration and the comparison with retrieved implants could help gaining deeper insight in the study of metal alloys related phenomena.

References:
**METAL-METAL WEAR PARTICLE CHARACTERIZATION AND BIOLOGICAL EFFECTS**

Isabelle Catelas, Ph.D

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**Introduction:** Wear particle-induced periprosthetic osteolysis remains a major cause of joint replacement failure. Metal-Metal (MM) Total Hip Replacements (THR)s have been developed as an alternative to the use of conventional Metal-Polyethylene (MPE) bearings that have been demonstrated to generate large volumes of PE particles, leading to bone resorption and implant failure. However, although the volumetric wear rate of MM bearings is much lower than that associated with conventional MPE bearings, biological concerns have raised due to the smaller size of the metal wear particles as well as the release of corrosion products. With an increasing need to implant hip prostheses in younger and active patients, a precise characterization of MM particles as well as a better understanding of the biological activity of these wear particles is critical in the attempt to modulate their clinical effects and to develop newer materials with greater wear and corrosion resistance.

**Characterization of MM wear particles:**

While the precise mechanisms by which wear particles induce periprosthetic osteolysis are still not fully elucidated and remain an active research area, the particle characteristics, such as composition, size, shape, and overall number (especially for those in the most biologically active submicrometer-size range) are known to play a role in the cell and tissue response.

When characterizing MM particles, differences in manufacturers, implant designs and alloys, as well as the use of various metal particle isolation and characterization techniques, have rendered direct comparisons between studies very difficult. An in vivo study by Doorn et al. using an enzymatic digestion of periprosthetic tissues reported that most particles were round, but a small portion were shard or needle-shape (1). Also using an enzymatic isolation protocol, newly developed and less damaging to wear particles compared with strong alkaline protocols previously used by others to isolate metal particles, Catelas et al. demonstrated that wear particles produced by MM implants in vitro and in vivo were in the nanometer size range, mostly round to oval but with some needle-shape, depending on the cycling period (for in vitro particles) and the implantation time (for in vivo particles) (2-4).

Most of the particles contained Cr and O but no Co, and were, therefore, most likely chromium oxides. The origin of these particles still remain under investigation but it was hypothesized that they were produced by wear of the passivation layer initially covering the implant surface and later replaced by an oxide layer (2-4). CoCrMo particles, which were fewer, may have originated from broken carbides (for the smallest and round ones) and/or the prosthesis matrix itself (for the needle-shape ones). These results corroborated the findings of Doorn et al., but differed to some extent from those reported in vitro by Brown et al. (5), showing exclusively round, and no needle-shape, Co-Cr particles. These discrepancies in particle shape and composition could be attributed to differences in the metallurgy of the prosthetic component alloys and loading parameters.

All studies, however, agreed that metal-metal particles were in the nanometer-size range with, therefore, a high specific surface area (surface area/mass), and subject to corrosion, leading to the release of metal ions into the surrounding areas. Therefore, the effects of metal ions also need to be considered when analyzing the biological response to these wear particles.

**Biological effects of MM wear particles:**

Histological studies of periprosthetic tissues from MM THR$s revealed the presence of an inflammatory reaction similar to that observed in MPE tissues, but the extent of this inflammatory reaction and the presence of foreign body type giant cells were much less important in MM tissues (1,6,7). This difference could possibly be explained by the overall smaller size of wear particles from MM bearings.

In addition to the potential of metal particles to induce osteolysis through macrophage activation as observed surrounding conventional MPE implants, a concern has emerged regarding the possibility of immunological delayed-type hypersensitivity responses with MM implants. This concern has risen from the observation of perivascular lymphocyte accumulations in tissues from failed MM implants (6,8). In some cases, the presence of plasma cells, B lymphocytes, and massive fibrin exudation that are not characteristic of a type-IV delayed-type
A hypersensitivity reaction was also reported (9). The overall reaction was then called aseptic lymphocyte-dominated vasculitis-associated lesion (ALVAL). These biological responses could be due to the release of metal ions that act as antigens and stimulate an immunological reaction when they form organo-metallic complexes with proteins. However, it is still unclear if the loosening of the implants can be linked to an increased reactivity of the lymphocytes to metal particles or ions (10). Very recently, Pandit et al. also reported the presence of pseudotumors characterized by an extensive necrosis of dense connective tissue, a focally heavy macrophage and lymphocytic infiltration, as well as the presence of plasma cells and eosinophils in some cases, surrounding MM surface replacements (11). The causes of these pseudotumors remain unknown but the authors raised the potential effects of wear particles as well as a possible hypersensitivity reaction. Although the incidence with which such immunological responses might cause the premature failure of a MM joint replacement is probably low, it is presently unknown.

Finally, concerns have also emerged that the metal wear particles may have cytotoxic effects through their dispersal throughout the body. Indeed, systemic dissemination of soluble and particulate corrosion products has been described and raises questions about potential genotoxicity. The presence of metallic particles in the liver and spleen has been reported (12) and higher ion levels have been measured in serum, urine, whole blood, and erythrocytes of patients with MM implants (13-15). These metal ions have theoretical, although not proven, risks related to carcinogenic and other biologic concerns, such as mutagenesis.

**Conclusion:** Because of the nature of MM particles (mainly chromium oxide particles in the nanometer size range), future biological studies should focus on the effects of these particles rather than just CoCrMo particles. As discussed above, previous studies showed that MM implant wear and corrosion can result in the production of wear particles and degradation products that can lead to a highly inflammatory biological response, leading to periprosthetic bone loss and aseptic loosening. The local and systemic effects of these wear particles and corrosion products also remain clinically significant issues. Efforts should continue to focus not only on developing and studying newer materials with greater wear and corrosion resistance and less biologic reactivity, but also on elucidating cellular and molecular mechanisms leading to periprosthetic osteolysis in order to identify new approaches for therapeutic intervention that would minimize the clinical impact.

**References**


**NOTES:**
Tribology of MM THA — Reason for Particles and Can Anything Be Done About It

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Metal-metal (MM) total hip arthroplasty (THA) arthroplasty, both stem type (ST) and surface replacement (SR), are made from wrought (ASTM F1537) or cast (ASTM F75) cobalt-based alloys each with about 0.24% carbon. The implants are manufactured with smooth surfaces, have specific radii of curvature and do not deviate much from being perfectly spherical (Fig.1).

Although cobalt-based alloys have the strength and fatigue resistance to avoid in situ structural damage, there are concerns regarding the wear particles causing osteolysis and other adverse biological responses (tissue toxicity, DNA damage, hypersensitivity). The present study considers attempts to quantify and reduce wear to reduce the risk of these problems. The purpose then is to explore the wear of MM THA, paying particular attention to in vitro measurement and mechanisms of wear. This is followed by explaining how wear can perhaps be reduced by fluid film lubrication and by a particular feature of the surface microstructure. Support for this reduction in wear is sought from simulator and retrieval studies. Although simulators provide the best in vitro assessment of wear, various pin-on-plate devices can also provide information more quickly and often with more precision. The wear mechanisms include abrasion, that can be observed with microscopy, tribochemical reaction layers and an influential surface microstructure. The retrieved nano-particles support the wear mechanisms to some extent. However, the real issue is the reduction of wear. A simple elastohydrodynamic lubrication model is developed that suggests a powerful way that geometry can help protect the surfaces [1]. Unfortunately, this lubrication cannot protect the surfaces at all times and may have inherent misrepresentations of the tribology. Stopping, dwelling and starting must cause some film breakdown and the surface carbides cause scratches that would drain a fluid film. Furthermore, the fluid rheology is not well understood when the films are relatively thin.

However, it can be strongly suggested that a large diameter, low clearance implant geometry (high effective radius) should promote some fluid film protection.

When surface contact does occur, Varano et al [2] showed that the amount of dissolved carbon in the alloy matrix could suppress a strain-induced transformation to a higher wearing HCP crystal microstructure at the surface. Although manufacturing processes have not been developed to achieve this alloy characteristic, there is also the suggestion that reducing contact stress with a large effective radius is also beneficial. Thus, a large effective radius (giving lower contact stress) has benefits to both lubrication mechanics and microstructural transformations.

To explore this approach, an effort was made to correlate both simulator and retrieval wear with the effective radius. For MM hip implants,
the wear appears to decrease with increasing effective radius (Fig. 2).

Although it is a little speculative to say this, it seems that MM hip implants benefit from large diameter and low clearance (large effective radius). Furthermore, if the amount of dissolved carbon can be increased, wear might be further reduced.

NOTES:

References:

INDICATIONS AND USAGE OF METAL-ON-METAL BEARING IN TOTAL HIP ARTHROPLASTY.

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Associate Professor, Head, Adult Reconstruction, University of Ottawa, Ottawa, Canada

INTRODUCTION: With the increasing demand being put on total hip arthroplasty combined with the increasing patient expectations to return/maintain high activity levels, metal on metal bearings are being used in an effort to maximize the longevity of the primary hip replacement. Although, metal on metal bearings haven been in clinical use for over twenty years with excellent results, metal hypersensitivity with associated osteolysis remains a concern as well as importance of acetabular component inclination especially with larger diameter femoral heads with monoblock acetabular components where excessive wear is being reported. This presentation will review the current applications of metal on metal bearing as well as associated complications.

CLINICAL RESULTS: Since 1984, over 200 000 MOM hip arthroplasties have been implanted worldwide. Using the Metasul hip with a 28mm head, Long et al found good medium term (5-11 year follow up) results in 96 hips. They had found only some calcar resorption in 6 hips and had revised one loose cup. Saito et al reported 99.1% survival at average 6.4 years with no cases of Osteolysis using the Metasul hip. Migaud et al compared a 28mm MOM hip with a matched control group using 28mm Ceramic-on-polyethylene. They found that at a minimum 5 year follow up the MOM hips fared better than Ceramic-on-polyethylene with osteolysis (9/39) and revision (7/39) occurring in the ceramic-on-metal group but not in the MOM group. Cuckler et al compared the results of 28mm to 38mm femoral heads in MOM hips and found that the dislocation rate was 2.5% v 0% respectively. Peters et al similarly found a 0% dislocation rate in 136 patients who had 38mm heads compared to 2.5 % (4 dislocations) in 28 mm heads. The reduced chance of dislocation that large head MOM hips offer mean fewer restrictions need be placed on the patient and a higher postoperative activity level can be achieved. This is especially relevant since return to recreational activities ranks high in terms of patient expectations after total hip replacement. In addition, studies have shown that increased activity levels postoperatively are associated with higher patient satisfaction rates. MOM hips have the added benefit for high activity patients of being able to withstand impact loading with no implant fractures being recorded.

MOM articulations have also facilitated the reintroduction of hip resurfacing with excellent short term results. On the acetabular side the relatively thin wall of the metal cup and the lack of keyholes means less bone stock is taken compared with a cemented polyethylene socket. Although, wear debris induced failures have been significantly reduced in hip resurfacing with the introduction of MOM, patient selection and proper surgical technique still remain key determinants of its clinical success.

Our current indications for either Stem Type metal on metal total hip replacement:
- Advanced Hip Arthritis
- Active patients less than 75 years of age
- Patients Not suitable for hip resurfacing
- Revision hip surgery with cementation of liner within pre-existing acetabular shell with deficient locking ring.

Metal Ion Release: Multiple clinical studies have documented the elevation in metal ions (cobalt and chromium) in serum, blood, erythrocyte and urinary levels as well as accumulation in abdominal lymph nodes, liver and spleen. Recent publications have pointed out the importance of proper acetabular component orientation (cup abduction angle less than 50 degrees) in terms of wear properties and metal ion release. A has been shown to be associated with higher wear and metal ion release. However, it is unclear how implant design in terms of diametral clearance as well as inner bearing diameter may influence the wear properties in terms of acetabular component position. More specifically, the inner bearing surface design of mono block acetabular shells are different than dose used with a modular acetabular metal liner. For example, some monoblock acetabular shells are less than a hemisphere varying from 180 to 164 degrees. In addition, some acetabular components have a thicker central pole which lateralizes the hip center of rotation and decreases the overall surface area.
for bearing contact\textsuperscript{18}. Thus these types of shell designs may have a narrower range of acceptable cup abduction being more susceptible to edge loading wear due to the smaller area of surface contact\textsuperscript{19}. In a recent paper, DeHaan et al\textsuperscript{19} reported that hip resurfacings with cup placed at greater than 55 degrees of abduction had significantly metal ion levels for both Co and Cr (Table 1) and that smaller femoral component sizes were found to be outliers in terms of ion release. When they examined specific designs of the acetabular component i.e. arc of cover or bearing surface contact, those implants with a lower arc of cover (164 versus 170 degrees) had significantly higher concentration of metal ions (cobalt & chromium) when placed at > 55 degrees of abduction (Table 2).

**Clinical Concerns**: Although rare, potential adverse reactions to metal particles needs to be considered and discussed with the patient. Willert et al\textsuperscript{21} described the Aseptic lymphocyte dominated, vasculitis associated lesion (ALVAL) or also known as LYDIA (lymphocyte dominated immunological answer)\textsuperscript{22} presenting as persisting hip pain with some cases associated with osteolysis. The incidence of this reaction was low (less than 0.3%) and may be linked to wear of the prosthesis. There has been concern about the use of MOM replacements in woman of childbearing age due to the unknown effects of metal ions on the unborn child. Brodner et al\textsuperscript{23} were not able to demonstrate ions crossing the placenta however a more recent study by Ziaee et al\textsuperscript{24} using more sensitive measurement techniques did demonstrate that ions crossed the placenta. With this in mind most would advocate that women should have their children before having a MOM hip or if they have had one, should not get pregnant until beyond the bedding in phase at 1-2 years.

### Table 1:

<table>
<thead>
<tr>
<th>ALL CUPS</th>
<th>Cobalt</th>
<th>Chromium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steep &gt; 55</td>
<td>9.8 µg/l (0.6 to 111.3)</td>
<td>9.7 µg/l (0.6 to 94.6)</td>
</tr>
<tr>
<td>Non Steep</td>
<td>2.4 µg/l (0.4 to 31.5)</td>
<td>3.6 µg/l (0.2 to 32.2)</td>
</tr>
</tbody>
</table>

### Table 2:

<table>
<thead>
<tr>
<th>Steep Cup (&gt; 55 degrees)</th>
<th>Cobalt</th>
<th>Chromium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implant of cover 164 degrees</td>
<td>10.2 µg/l (0.8 to 111)</td>
<td>10.4 µg/l (0.6 to 94.6)</td>
</tr>
<tr>
<td>Implant of cover 170 degrees</td>
<td>2.1 µg/l (0.6 to 6.4)</td>
<td>3.0 µg/l (0.9 to 7.6)</td>
</tr>
</tbody>
</table>

**Reference:**


Session: **Materials and Methods in Trauma Surgery**

in presentation order:

G. **Taeger**, Essen, Germany  
*Clinical Relevance of Corrosion of Thin Layers in Joint Arthroplasty.*

B. **Hußmann**, Essen, Germany  
*Does Corrosion Matter in Titanium Plates?*

M. **Weuster**, Essen, Germany  
*A next generation of implant material or just another piece of metal: Summary of experimental data of P2000.*
Clinical Relevance of Corrosion of Thin Layers in Joint Arthroplasty.

G. Taeger

Essen, Germany
Steel and titanium osteosynthesis implants are still competing for the best economic and medical biological material. While steel has been regarded critically for years because of possible corrosive surface changes, problems in titanium concerning the material such as cold shuts come to the fore more and more, also in the modern angle stable implants. The aim of our study was to find out if titanium despite its oxidation ability is submitted to corrosive changes and a measurable material abrasion takes place.

In a standardised fracture model of rabbit tibia (NZLWR) 15 animals were treated with plate osteosynthesis of steel (316L) and titanium (cpTi) respectively. The osteosynthesis was made with a 1mm gap to guarantee the strain of the implant in the plate/screw interface. The animals were fully strained postoperatively and were sacrificed after six weeks. The implant materials were removed and cleaned in ultrasonic bath. The analysis of corrosive changes and material abrasion (fatigue) was carried out by means of stereo macroscope (Leitz) and electron microscopy (SEM, DSM962). Results were evaluated according to the classification of Cook. Additionally, the tissue samples of the implant bed were analysed for their content of material components by means of ICP-OES.

In the steel as well as in the titanium implants corrosive changes appeared. Classification of the corrosive changes by means of stereo macroscopy was easier in the steel implants due to colour differences. According to Cook, 65.9 points were determined for 316L and 16.6 for cpTi. In contrast, in cpTi a significantly higher material abrasion than in 316L (8.2 in 316L versus 46.7 in cpTi; p<0.001) was observed. Correspondingly, in the ICP-EOS the concentration of titanium components was observed to be a factor 8 higher compared to 316L. This emphasizes the susceptibility of titanium as material for osteosynthesis implants to corrosion and fatigue in the strained model.

The study shows that titanium in the strained situation of a plate osteosynthesis provokes corrosive changes and material abrasion. Furthermore, the local tissue is loaded with metal products. These results relativise the highly appreciated significance of titanium for mechanically strained osteosynthesis implants. These findings can result in the preference of steel implants due to the evaluation of the difficulty of cold shuts in angle stable plates.
A next generation of implant material or just another piece of metal: Summary of experimental data of P2000.

M. Weuster

Essen, Germany
Session: **Materials and Methods in Trauma Surgery and Orthopedics**

in presentation order:

D. **Tarnita**, Craiova, Romania  
*Modular adaptive bone plate based on intelligent materials.*

B. **Levine**, Chicago IL, USA  
*Porous Metals in Orthopedic Applications*

S. **Utzschneider**, Munich, Germany  
*Wear of contemporary knee designs and materials*

S. **Landgraebber**, Essen, Germany  
*DNA-repair mechanisms in Aseptic Loosening after Total Hip Replacement*
MODULAR ADAPTIVE BONE PLATE BASED ON INTELLIGENT MATERIALS

Tarnita Daniela¹, Tarnita D.N.², Bizdoaca, N.³, Popa, Dr.⁴

¹, ⁴) University of Craiova, Faculty of Mechanics, Romania
²) University of Medicine and Pharmacy, Craiova, Romania
³) University of Craiova, Faculty of ACE, Romania

INTRODUCTION: Applications of Shape Memory Alloys to the biomedical field have been successful because of their advantages over conventional implantable alloys, enhancing both the possibility and the execution of less invasive surgeries. Super-elastic NiTi has become a material of strategic importance as it allows to overcome a wide range of technical and design issues relating to the miniaturization of medical devices and the increasing trend for less invasive and therefore less traumatic procedures.

- the current mechanical devices used in orthopedics lose some of their mechanical characteristics after some time (especially elasticity, which should ensure a constant tension that is mandatory for the correct anatomical healing of the fractured bones);

- the process of fracture healing has a particular dynamic, which imposes the necessity of particular progressive tension or discharge to improve the recovery time, depending on the normal structure and function of the bone;

- to improve the healing process, the fractured parts have to be in permanent contact in order to ensure the proper conditions to develop bone calluses.

- a minimally invasive surgery ensures protection and improves bone recovery.

The solution to these problems is the Modular Adaptive Implant. This device will partially...
discharge the tensions in the fractured bones (the fractured parts still need to be tensioned to allow the formation of the callus) improving the recovery time and the healing conditions.

The proposed intelligent device is a modular bone plate, with modules made out of Titan and the staples made out of Nitinol. The shape memory staples, in their opened shape, are placed in the special places build in to the modules. Through heating, this staple tends to close, compressing the modules and determining the translation of the modules. In this way, the separated parts of bone are compressed.

The force generated by this process accelerates healing and reduces the time of bone recovery. Moreover, these modules allow little movement in the alignment of the fractured parts, reducing the risks of wrong orientation or additional bones callus.

**RESULTS:** The modules and the staples are designed in SolidWorks. We used ANSYS software for discretisation and Visual Nastran for modular plate simulation.
Applications of Porous Metals in Orthopaedic Surgery

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The development of porous metals and coatings is a historic achievement that has led to major advances in the field of orthopaedic surgery. In the past, the majority of implants have been fabricated utilizing traditional metals (cobalt-chromium, titanium and stainless steel alloys) and coatings (fiber metal, sintered beads, plasma spray and cancellous structured titanium). Despite excellent clinical results utilizing these materials, there remains some inherent biomechanical and structural limitations to these traditional metals/coatings. In light of these relative short-comings, a new generation of porous metals/coatings have been introduced to improve upon clinical results and expand operative indications for use of these new materials.

Tritanium (Stryker, Mahwah, NJ), Regenerex (Biomet, Warsaw, IN), Stiktite (Smith and Nephew, Memphis, TN), Gription (Depuy, Warsaw, IN) and Trabecular Metal (Zimmer, Warsaw, IN) are examples of the latest generation of porous metal/coatings available for orthopaedic surgery. The microscopic appearance of these metals is similar to that of cancellous bone and all contain complex nanostructures. The relative open-cell structure of these new materials affords several intriguing properties, including; high volumetric porosity (60-80%), low moduli of elasticity and high-friction surface characteristics. Current orthopaedic implants are available for applications in spine, hip, knee, shoulder and ankle reconstructive procedures, with successful early results being reported. Adaptations for use as cartilage replacement scaffolds and for bone graft substitution are being investigated at this time. This new generation of porous metals/coatings is derived mainly from titanium and tantalum compounds both of which are relatively inert transition metals. Each maintains a high level of biocompatibility in vivo as evidenced by their use in current orthopaedic implants, pacemaker electrodes, cranioplasty plates and as radiopaque markers. Titanium and tantalum have a well-documented bioactive nature, forming a bone-like apatite coating in vivo. This high affinity for bone and fibrous tissue ingrowth has extended the limits of these new porous constructs for use in megaprostheses, glenoid and ankle fixation, patellectomy and patella salvage. Although these new porous metallic options are in their early stages of evolution, the initial clinical data and basic science studies support their use as an alternative to traditional implant materials. The following represents a detailed review of the history, biomechanical properties and applications in orthopaedic surgery for traditional and modern porous metals/coatings.
Wear of contemporary knee designs and materials

Sandra Utzschneider, Alexander Paulus, Norbert Harrasser, Jean-Christophe Datz, Christian Schröder, Wolfgang Plitz, Volkmar Jansson

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Orthopaedic Department, Laboratory for Biomechanics and Experimental Orthopedics, Ludwig-Maximilians-University, Großhadern Medical Center, Munich, Germany

INTRODUCTION: Periprosthetic wear plays a central role in osteolysis and loosening of the implants. The osteolytic reaction is influenced by dose, size and shape of the wear particles, so that articulating surfaces with low wear rates and biologically less active particles are required. Crosslinked polyethylene (XPE) was developed to reduce wear in both total hip and knee prostheses. In addition, in the knee a range of design and kinematic variables have to be considered as they can markedly influence wear regardless of the type of polyethylene used.

Therefore, the purpose of this study was to determine the wear rates of different current knee designs and bearing materials and analyze the size and morphology of the polyethylene particles.

MATERIAL AND METHODS: In a knee-joint-simulator (Stallforth-Ungethuem) 4 XPEs (1 sequential irradiated and annealed, 3 different remelted types, fixed- and mobile-bearings) and 2 UHMWPE-inserts (fixed- and mobile-bearings) were tested (ISO) with the appropriate femoral and tibial component recommended from the particular manufacturer (all the fixed-bearing inserts of XPE are actual products, the mobile-bearing insert of XPE is not an actual product nor is it ever intended to be). The gravimetric wear rates (mg/year) were measured (5mill. cycles), the wear mechanism was analyzed by SEM. 100,000 particles were analyzed by SEM (20nm-nucleopore-filter; acid digestion method; ISO).

RESULTS: All the inserts showed traces of abrasion, scratching and wear polishing. XPEs produced lower wear rates (min 0.6 – max 4.3mg/year; p<0.05) than UHMWPEs (min 8.4 – max 8.5mg/year) without fatigue reactions. There were no differences in the wear rates between fixed-and mobile-bearings (p>0.05). The sequentially irradiated and annealed insert showed the lowest wear rate (0.6mg/year; p<0.05) overall.

For all the designs, the wear was predominantly smooth, granular and irregular with few fibrillar particles, more than 85% of the particles were submicron. All the types of polyethylene used in this study showed similar size distributions (mean ECD; 0.39 ± 0.37μm - 0.42 ± 0.44μm for the UHMWPEs and 0.33 ± 0.23μm - 0.46 ± 0.46μm for the XPE). The particle size was independent of the radiation dose. The wear generated by the mobile-bearings (XPE and UHMWE) had a higher (p<0.05) percentage fraction of particles above 1μm in size (ECD) than all the fixed-bearings.

DISCUSSION: All crosslinked tibial inserts, fixed- as well as mobile-bearings, show statistically significant (p<0.05) reduced wear rates without any fatigue reactions. The fixed-bearing sequential irradiated and annealed insert has the lowest wear rate overall (p<0.05). XPEs (fixed- and mobile-bearings) and UHMWPEs have similar wear particles in shape. We could not confirm that “crosslinking” itself leads to smaller particles. Fixed-bearing XPE and UHMWE show slightly smaller particles than the mobile-bearings. Further investigation is needed to determine if the differences in size seen in this study lead to different biological responses.
DNA-repair mechanisms in Aseptic Loosening after Total Hip Replacement

Stefan Landgräber¹, Marius von Knoch¹, Franz Löer¹, Kurt Werner Schmid², Martin Totsch²

¹Department of Orthopaedics, University of Duisburg-Essen, Pattbergstrasse 1-3, 45239 Essen, Germany
²Institute of Pathology and Neuropathology, University of Duisburg-Essen, Hufelandstrasse 55, 45122 Essen, Germany

Particle-induced osteolysis is a major cause of aseptic loosening after hip joint replacement. While most authors have focused their interest on the cytokine release from macrophages initiated by wear particles and the following activation of the osteolytic cascade, we are interested in cellular regulation and repair mechanisms. To elucidate if the potency of the DNA-repair mechanisms correlates with the survival of joint implants we compared the immunohistochemical ERCC1 expression in capsules and interface membranes of patients with loosening of a hip replacement in the first ten years after implantation with those in patients with late loosening. The level of ERCC1 reaction in the specimens taken from patients with early aseptic loosening was clearly lower in comparison with those from patients undergoing exchange hip arthroplasty later than ten years after surgery. In conclusion, the analysis of wear particle-induced ERCC1 activity allows the identification of a group with a very high risk for early aseptic hip loosening.
Postersession (alphabetical order)

T. Aleksyeyeva (Kiew, Ukraine)
BIOCOMPATIBILITY OF MULTI WALL CARBON NANOTUBES AND NANOCOMPOSITES

M. Bezuglyi (Kiew, Ukraine)
STRUCTURE PECULIARITIES OF SPHERICAL POLYMER BRUSHES DEXTRAN-GRAFT-POLYACRYLAMIDE

E. Boanini (Bologna, Italien)
CHEMICO-PHYSICAL CHARACTERIZATION OF GELATIN FILMS MODIFIED WITH OXIDIZED ALGINATE

E. N. Bol'basov (Tomsk, Russische Föderation)
USE OF COMPOSITION MATERIALS ON THE BASE OF COPOLYMER OF THE TETRAFLUOROETHYLENEWITH VINYLIDENE FLUORIDE FOR NEEDS OF TRAUMATOLOGY AND ORTHOPAEDICS

M. Cieślik (Stockholm, Schweden)
SURFACE INVESTIGATIONS OF A HIP JOINT IMPLANT AFTER 10 YEARS OF USE IN VIVO

J. Czeczor
IS THE PIG AN IDEAL LARGE ANIMAL MODEL FOR CARDIOVASCULAR DISEASES?

J. Czeczor
DIFFERENTIATING ADIPOCYTES CAUSE ENHANCED MIGRATION OF PRIMARY ENDOTHELIAL CELLS

A. Dannan (Witten-Herdecke, Deutschland)
IMAGING OF PERIODONTIUM DERIVED STEM CELLS AGGREGATION ON ZIRKONOXID SURFACES

A. Ewald (Würzburg, Deutschland)
EFFECT OF COLD-SETTING CALCIUM- AND MAGNESIUM-MATRICES ON PROTEIN EXPRESSION IN OSTEOBLASTIC CELLS

M. Granseier (Dortmund, Deutschland)
VIRTUAL SURFACE ANALYSIS OF THE MEMBRANE MORPHOLOGY OF INFECTED VITAL MACROPHAGES

C. Greulich (Bochum, Deutschland)
BIOLOGICAL ACTIVITIES OF SILVER NANOPARTICLES ON HUMAN MESENCHYMAL STEM CELLS (HMSCS)

A. Hubina (Kiew, Ukraine)
POLYURETHANE BIOMATERIALS FOR SORPTION THE PHENOLS FROM THE WATER SOLUTIONS

C. Hübsch (Garbsen, Deutschland)
OBSERVATION OF HYDROTHERMALLY INDUCED PHASE TRANSFORMATION OF YTTRIA-STABILIZED ZrO2-CERAMICS FOR DENTAL APPLICATIONS

Y. Kamenchuck (Tomsk, Russische Föderation)
INFLUENCE OF CHITOSAN ADDITIONS ON OSTEOGENIC PROPERTIES OF CALCIUM PHOSPHATE COATING OBTAINED BY MEANS OF MICRO ARC OXIDATION

I. Khlusov (Tomsk, Russische Föderation)
MODIFICATION OF BONE TISSUE ENGINEERING IN VIVO BY RGD PEPTIDES DEPOSED ON NANOSTRUCTURED CALCIUM PHOSPHATE COATING

M. Kirsch (Essen, Deutschland)
PERFLUOROCARBON-FILLED POLY(LACTIDE-CO-GYLCOLIDE) CAPSULES AS POTENTIAL ARTIFICIAL OXYGEN CARRIERS FOR BLOOD SUBSTITUTES
V. Krutsko (Minsk, Weißrussland)
FLEXIBLE FIBROUS BIOMATERIALS MODIFIED BY HYDROXYAPATITE

M. Lange (Essen, Deutschland)
RH-BMP-2/POLY-(D,L)-LACTIDE-MEMBRANES FOR BONE TISSUE ENGINEERING

O. Lazarenko (Kiew, Ukraine)
ATOMIC FORCE MICROSCOPY FOR PREDICTION THE VASCULAR TISSUE REACTION (EXPERIMENTAL STUDY)

B. Levine (Chicago, USA)
EARLY CLINICAL AND RADIOGRAPHIC RESULTS OF POROUS TANTALUM PRIMARY HIP AND KNEE PROSTHESES

H. Lipinski (Dortmund, Deutschland)
A VIRTUAL 3D SCENE CONTAINING INTERCELLULAR CONNECTIONS OF VITAL BONE CELLS

S. Lüers (Essen, Deutschland)
DEVELOPMENT OF NOVEL MICRO- AND NANO-STRUCTURED TITANIUM SURFACE FOR DENTAL IMPLANTS BY ACID ETCHING

S. Madenci (Essen, Deutschland)
BIOLOGICAL ACTIVITY OF ULTRAHYDROPHILIC AND HYDROPHOBIC IMMOBILIZED RHBM-2 ON A TITANIUM SAND BLASTED AND ACID ETCHED SURFACE

V. Martínez (Saragossa, Spanien)
IMPROVED WEAR PERFORMANCE OF ULTRA HIGH MOLECULAR WEIGHT POLYETHYLENE COATED WITH HYDROGENATED DIAMOND LIKE CARBON

M. Martínez -Morlanes (Saragossa, Spanien)
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M. Meißner (Essen, Deutschland)
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Objectives: Various biomedical applications of carbon nanotubes have been proposed in the last few years leading to the emergence of a new field in diagnostics and therapeutics (1). Use of nanomaterials, nanoparticles and nanocomposites for biomedical purposes constitutes a burgeoning new field called nanomedicine. The toxicological and pharmacological profile of such carbon nanotube systems have to be determined prior to any clinical studies undertaken. Experiments in vivo (1) show that carbon nanotubes (CNT) do not cause complication, and in literature (2) suggest to use the CNT as delivery component for vaccine. Modification of CNT leads to reduction of bundling/aggregation of individual tubes through van der Waals forces and to increasing of uniform distribution of CNT in medium. Polymers filled (extended) with CNT show the extraordinary physical, mechanical and electronic property (4). Present study describes the in vivo study of the living body response to the CNT purified aggregates and nanocomposites: polypropylene (PP) and PTFE filled with CNT in different concentration. As it is known that the surface chemistry is the principal factor in body reaction to the foreign material, the changes in surface chemistry in comparison with initial polymers were studied with AFM. Also there was studied the influence of the solution composition (electrolytes and biomolecules) on the reduction of bundling/aggregation forces of individual CNT.

Experiment: CNT were obtained and characterized as previously described (5). CNT had average diameter 10-20nm, specific surface, determined by argon desorption, was 200-400 m²/g, bulk density was at range 20-40 g/dm³. Ash of nonpurified CNT was 6-20%, in purified ones about 1%. The XR analyze of CNT gave d₀₀₂ parameter for graphitelike structure in interval of 0.3431-0.3451 nm. Major X-ray reflection broadening was due to low interval of coherent scattering and microstress. According to XPS data CNT’s surface contained about 0.6% at of oxygen, and their relative distribution according to the different types of oxygen containing centers, that was calculated by C 1s spectra decomposition, were following: phenols and alcohol – 49.1%, ketonic and kinone -17.2%, carbonic or/and absorbed CO, CO₂ -16.5%. The samples of nanocomposites PP-CNT with 0.05, 1.0, 3.0 and 5.0 w% concentration of CNT, PTFE-CNT with 15 w% ones, and initial polymers were selected for in vivo study. Modification CNT in different solutions of electrolytes and biomolecules leads to reduction of bundling/aggregation between individual nanoparticles (pic.2). Photo shows the strong affect of biomolecules on the reduction of van der Waals forces. The strongest effect observed in the case of lower biomolecular weight (albumin 60 kD vs heparin 14 kD).

The results in table 1 shows that the value of forces of tip interaction with nanocomposites surface PTFE-15% CNT in all cases was lower than ones with “initial” PTFE. According to these findings the “reactivity” of nanocomposites may be less in comparison with initial PTFE in living system. The samples were implanted intra muscular to experimental animals. In 4 weeks after operation the samples with surrounding tissue were excised for the further histological study and investigation of the samples’ surfaces. The organism response to the foreign body was evaluated by thickness of fibrous-connective capsule that formed around the samples. Histological study shows that the recipient’s tissue reaction to implanted samples was various. In the case of initial PTFE there was formed thick and solid capsule (362.6 ± 24.7 µm) with a lot of artifacts. The capsule thickness around composites was 58.4 ± 8.2 µm. In surrounding tissue the artifacts were also observed, but to a lesser degree in comparison with initial PTFE. The biocompatibility properties of nanocomposites are not linear function of CNT concentration in polymer matrix.
The biochemical data shows that inflammation in surrounding tissue in case of samples with 3 and 5% CNT were local.

**Conclusion:** CNT in polymer matrix strongly modify the nanocomposites surface chemistry and influence on their affinity with living body.

**Reference:**

2. L.Lacerda, A.Bianco, M.Prato, K.Kostarelos./ Carbon nanotubes as nanomedicines:
Introduction: The polymer-brush theory and some experimental works with non-linear polymers open the future trends for obtaining a novel type of polymer materials. Such substances possess unique properties due to their possibility to undergo the controlled structure transformations: change in shape, size, etc. A great number of variable parameters such as initial polymer architecture, average degree of polymerization, nature and flexibility of backbone and grafts, distance between grafts, etc. can influence the formation of nanostructure and determine the final properties of such compounds.

Water-soluble Dextran-graft-Polyacrylamide (D-g-PAA) copolymers consist of two non-toxic and biocompatible components that are widely used for biomedical application. These materials should be perspective as multifunctional nanoplatforms or matrices loading active components inside thanks to more compact internal structure of polymer brushes in comparison with linear polymers.

Materials & Methods: Two series of D-g-PAA copolymers with polysaccharide backbone having different molecular weights (\(M_w=20\ 000\) and \(M_w=70\ 000\)) were synthesized. Ce(IV)/HNO\(_3\) redox system was used as polymer initiator. The average number of grafting sites per backbone molecule depends on the ratio of ceric ion concentration to Dextran \([1]\). The ratio of mol Ce(IV) to mol Dextran was equal to 5, 10 and 15. The amount of monomer AA was kept the same for all syntheses. The procedure of samples production and purification was described in \([2]\).

Results & Discussion: In water solution Dextran macromolecules possess conformation of coil with cooperative systems of internal H-bonds inside. Grafting PAA-chains onto Dextran occurs on surface of polysaccharide coil. The content of Dextran component in copolymers is less 3%. Thus, the D-g-PAA copolymers synthesized are star-like ones. They can be considered as spherical brushes \([3]\) with Dextran core and PAA-corona.

As it was predicted in the theoretical work \([3,\ 4]\) the compactness of polymer brushes depends on the distance between grafts and their conformation. The “factor of compactness” that is expressed as \(R_z^{2}/M\) \([4]\) has been analyzed for two series of copolymers and is represented in Table. When value of \(R_z^{2}/M\) is lower, the compactness is higher. The compactness depends on the grafts number as well as the grafts conformation.

LS investigations have shown that all samples have high molecular weights (\(M_w\)) and are in good agreement with viscometric data (Table 1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>(M_w\cdot10^{-6})</th>
<th>(R_z^{2}/M\cdot10^{-3})</th>
<th>([\eta]),g/dl</th>
<th>(N_{\text{Dex}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>D20-g-PAA5</td>
<td>3.65</td>
<td>6.33</td>
<td>3.4</td>
<td>25</td>
</tr>
<tr>
<td>D20-g-PAA10</td>
<td>2.22</td>
<td>4.87</td>
<td>2.0</td>
<td>12</td>
</tr>
<tr>
<td>D20-g-PAA15</td>
<td>1.08</td>
<td>3.22</td>
<td>1.2</td>
<td>8</td>
</tr>
<tr>
<td>D70-g-PAA5</td>
<td>3.11</td>
<td>3.15</td>
<td>2.75</td>
<td>86</td>
</tr>
<tr>
<td>D70-g-PAA10</td>
<td>2.43</td>
<td>2.50</td>
<td>2.1</td>
<td>43</td>
</tr>
<tr>
<td>D70-g-PAA15</td>
<td>2.29</td>
<td>2.33</td>
<td>1.9</td>
<td>29</td>
</tr>
</tbody>
</table>
While compare the samples of D70-g-PAA copolymers with D20-g-PAA ones having close molecular mass, we have ascertained that the values of $R^2/M$ for D20-g-PAA copolymers are higher (Table), therefore their compactness is lower. It is conformed our previous X-Ray results on copolymer internal structure for similar systems with 6 grafts [2]. The distance between grafts conditions the conformation of PAA-grafts: in the case of D20-g-PAA the scattering curve resembles closely that of a worm-like chain. The PAA-grafts of D70-g-PAA copolymers display a kind of “mushroom” conformation, which is more compact than “worm-like”. Also, the values of the z-averaged radius of gyration, $R_z$, are higher for linear PAA of the similar molecular weight as for graft copolymers. These experimental results agree with theoretical prediction on the compactness of polymer brushes [1].

Thus, LS and viscometry investigations have shown that all samples possess expanded conformation in aqueous solution. The internal structure of D-g-PAA copolymers is depended upon the distance between grafts of PAA-chains. Compactness of D20-g-PAA copolymers are lower in comparison with D70-g-PAA samples due to the different conformation of PAA grafted chains: “mushroom” conformation for D70-g-PAA and “worm-like” for D20-g-PAA samples.

These copolymers can be basis for multifunctional nanoplatforms with different acceptance of functional groups inside for loading active biomedical components. The local concentration of functional groups can be regulated through the structure peculiarities.

References:


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CHEMICO-PHYSICAL CHARACTERIZATION OF GELATIN FILMS MODIFIED WITH OXIDIZED ALGINATE

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Gelatin is obtained by thermal denaturation or physical and chemical degradation of collagen. Since gelatin is soluble in aqueous solution, gelatin materials for long-term biomedical applications must be submitted to cross-linking, which improves both the thermal and the mechanical stability of the biopolymer [1]. Chemical crosslinking agents, such as glutaraldehyde and isocyanates, are quite often toxic, whereas oxidized mono-, di- and polysaccharides have been suggested as possible non-toxic agents. Alginate, a natural polysaccharide extracted from seaweed, has been extensively investigated in a variety of biomedical applications. Furthermore, an important feature of alginate is its gelation in the presence of divalent cations, such as Calcium.

We have investigated the chemical physical properties of gelatin-alginate films. In order to improve the chemical interaction between gelatin and alginate chains, alginate was previously submitted to an oxidation process through reaction with periodate [2]. Type A gelatin films were prepared from a 5%, 10% and 15% aqueous gelatin solution. Films were obtained after water evaporation at room temperature. Gelatin-alginate films were prepared by adding to gelatin solution solid oxidized alginate at different concentration (1% and 3% w/w with respect to gelatin weight) and in the presence of Calcium ions. The results indicate that oxidized alginate can be successfully utilized to crosslink and stabilize gelatin films. In agreement with the low concentrations of ADA, the extent of crosslinking of the gelatin films is modest, reaching a maximum value of 23%. Nonetheless, the presence of ADA significantly reduces the degree of swelling and gelatin release in solution, and it improves the mechanical properties and the thermal stability of the films as put into evidence by DSC investigation and supported by X-ray diffraction results. Addition of Ca\(^{2+}\) ions can be used to further modulate the chemico physical properties of the films.

References:

1. Bigi, G. Cojazzi, S. Panzavolta, N. Roveri, K. Rubini Biomaterials 22 (2001), 763-768
2. Balakrishnan, A. Jayakrishnan Biomaterials 26 (2005), 3941-395
Introduction: In recent years new direction connected with producing of composites made of synthetic polymers and inorganic disperse particles for long bones treatment was developed. New hybrid materials with up to 300µm thickness have unique properties combination: high adhesion to metallic substrates, high tension strength and elasticity, control of osteoinductive and osteoconductive properties over a wide range [1]. Effect of such materials is based on polymers capacity to act as binding material and calcium phosphates ability to regulate bone tissue growth. We have made an attempt to use copolymer of tetrafluoroethylene with vinylidene fluoride as binding element. Copolymers of this line are used in medicine as nonresorbable suture material and endoprothesis for abdominal plastic when curing hernias.

Materials and Methods: Solution of copolymer of tetrafluoroethylene with vinylidene fluoride was prepared by means of dissolution of copolymer powder in acetone. Then hydroxyapatite (HA) powder dispersion was added to copolymer solution until reaching necessary system viscosity. Morphology of the surface of obtained composite coatings on titan substrate were studied by means of raster electron microscopy method (REM) on the Philips SEM 515 unit (fig. 1). Samples toxicity in vitro was estimated according to ISO 10993 on 24-hour cell culture of mice marrow. Biocompatibility and osteoinductive ability of composites were estimated in vivo in 20 Balb/c line male-mice with the mass of 18-21g. Discs with preliminarily applied marrow column extracted from animal femoral bone in the DIME M (ISN) medium with 10% of embryonic calf serum were implanted into mice subcutaneously. In 1.5 months animals were devitalized by means of ether anaesthesia, discs were taken out; stained sections of grown tissues were prepared und exposed to histological analysis [2].

Results: As shown on Fig. 1, composite surface has difficult pore structure consisting of HA particles with less than 1-2 µm dimensions connected with each other. Under in vitro and in vivo investigation of biomedical samples local and common toxic and inflammatory reactions were not revealed. Probability of bone tissue formation was not less than 75%. Histological section studies showed bone up- and ingrowth (fig.2).

Discussion: Studies carried out by us show the possibility in principle of using of calcium phosphate composite coatings with copolymer of tetrafluoroethylene and vinylidene fluoride in traumatology and orthopaedics.

References:

Surface Investigations of a Hip Joint Implant After 10 Years of Use in Vivo

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Due to the large increase in lifespan, the significance and range of implantations is dramatically increasing. Despite the importance relatively few studies on used implants are reported in the literature.

The aim of this paper is to report on the examination of the surface of the metallic part of an acetabular socket of a hip joint implant, using several classical material science methods, such as: scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDXS), X-ray powder diffraction (XRD) and X-ray fluorescence spectroscopy (XRF). The investigated socket was a press-fit type cup, made of a Ti-Al-V alloy, with self-cutting thread with a surface covered by plasma sprayed porous titanium (BIOMET). To retain the unchanged surface state the implant was only cleaned with water and rinse with ethanol after removal from the body. The reason for its removal from a 60-years old woman was a wear-out of the polyethylene part of the acetabular socket.

The photography of the investigated medical device (titanium alloy socket which was fastened by screwing into the bone without any polyethylene insert) is shown in the Figs. 1-3. The arrows indicate places from where SEM observations were done. The performed analysis revealed the in vivo changes in the implanted material after 10 years. SEM observations demonstrate relatively small changes of the surface in places where the socket did not contact directly with the bone (Fig. 1), whereas in places with intimate contact with the bone, a characteristic island-like deposit was formed (Fig. 3).

The elements in the deposit, were identified by using EDXS and XRF methods and it was found to consists of phosphorus, oxygen and calcium. X-ray diffraction of the deposit reveals that it has a stoichiometry close to that of hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_{6}(\text{OH})_2$. The main constituting elements of the socket surface without any deposit were titanium, aluminum and vanadium, the elements of the titanium alloy.

It can be concluded that direct interaction with the bone and the plasma sprayed surface preparation of the socket surface are the crucial factors for the precipitation of calcium phosphate. The results show also that a bone has succeeded grown into the implant porous surface.
Is the Pig an Ideal Large Animal Model for Cardiovascular Diseases?

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The most common intermediate step between the small, rodent animal model and first human clinical trials is a large animal model. In case of cardiovascular diseases, the pig is the state of the art due to its resemblance to human physiology.

In atherosclerosis studies, the endothelium is of major interest since this inflammatory disease affects arteries by plaque formation due to degeneration of lipids and calcification of the vessel wall. To understand the pathogenesis, the molecular background of the gene expression pattern in endothelial cells (ECs) must be clarified. A wide range of key molecules involved has been identified so far. Amongst these are endothelin (ET)-1 and platelet endothelial cell adhesion molecule (PECAM)-1 as they are involved in the maintenance of blood pressure and cellular adhesion.

A good animal model should contain suitable markers in similar ratios as found in humans. In this study, primary porcine aortic endothelial cells (PAECs) were isolated and investigated for EC specific surface marker on mRNA level and compared for differences in human microvascular endothelial cells (HMECs). One of these marker was endothelin, which has a key role in vasoconstriction and vascular homeostasis and thus is involved in vascular diseases of several organ systems including the heart. Interestingly, a significantly higher gene expression of endothelin-1 was found in PAECs compared to HMECs, whereas the expression of other factors such as PECAM-1 was found to be equal.

More factors need to be investigated in order to assess the quality of the model. Furthermore, it has to be evaluated if these findings are similar in the domestic and the inbred minipig to draw final conclusions on the quality of the pig as large animal model for cardiovascular diseases. This is of major importance since especially the pig is not only used as a large scale animal but also discussed as heart donor for xeno transplantation approaches in the future.
faDifferentiating Adipocytes Cause Enhanced Migration of Primary Endothelial Cells

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Migration of endothelial cells is a key event in angiogenesis. It is involved in processes such as wound healing and is dysregulated under pathological conditions like diabetic vasculopathy or atherosclerosis, two major sequelae in obese patients with diabetes type 2.

The developing adipositas is a major factor contributing to endothelial dysfunction, which can initiate atherogenesis since the adipose tissue is an endocrine organ, secreting a variety of adipokines. Novel studies have shown that also the differentiating adipocyte is highly metabolically active.

Mesenchymal stem cells (MSCs) are multipotent cells, which are capable to differentiate into the adipogenic lineage. In this study we used the supernatant of MSC-derived differentiating adipocytes to investigate its effect on primary bovine aortic endothelial cells (BAECs) as an atherosclerosis model.

We found that BAECs showed a higher migration rate in presence of the conditioned medium, which was independent of proliferation. On the molecular level, MMP9 was upregulated in the stimulated primary endothelial cells. This emphasizes the data that already the premature adipocyte is a contributing factor in early atherosclerosis development since it is known that matrix metalloproteinases (MMP) contribute to endothelial migration.

Taken together, the combination of differentiating MSCs and primary BAECs present a promising early atherosclerosis model which reflects in vivo conditions. Here, all stages of adipocyte differentiation, adipokine composition and their effects on the endothelium can be investigated in detail, opening new avenues for basic research and new drug testing for diabetes type 2 associated atherosclerosis.
Imaging of Periodontium-derived Stem Cells Aggregation on Zirkonoxid surfaces

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**Objectives:** Stem cell-loaded implants are becoming increasingly important in biomedical research, particularly in dental tissue regeneration techniques. Cellular responses depend on topographical properties of the biomaterial at the nanometer scale, and on the structures of biomaterial surfaces. However, stem cell-biomaterial interactions still poorly understood. The aim of this study is to give primary imaging of the aggregation ability of human Periodontium-derived Stem Cells (hPdSCs) when coated on different artificial materials.

**Methods:** In a serum-free culture medium containing Fibroblast Growth Factor (FGF-2; 20ng/ml, Chemicon, Hofheim, Germany) and Epidermal Growth Factor (EGF; 20ng/ml; R&D Systems, Wiesbaden, Germany), hPdSCs were allowed to proliferate for 4 days. Convex-shaped 7×7mm² zirkonoxid surfaces were separately incubated in the hPdSCs suspension for 3 days at 37°C and 5% CO2-humidified atmosphere. Thereafter, the materials were seeded onto self-constructed glass chambers, washed by Phosphate Buffered Saline (PBS), stained with CellTracker™ Green and then preserved again for 30 minutes at 37°C and 5% CO2-humidified atmosphere.

**Results:** As in normal culture, images taken by optical light microscope showed the ability of hPdSCs to form dentspheres on top of all the material surfaces. Images taken by confocal laser microscope clarified the general adhesion picture of hPdSCs. However, an exact detection of the cell-surface region topography was not possible.

**Conclusion:** According to these primary results, it could be stated that hPdSCs showed initial ability to reside and adhere on zirkonoxid surfaces. The general form of hPdSCs on those materials has been shown to be clusters/dentspheres. Although the adherence of hPdSCs seemed not to be stable 100%, their adherence ability on zirkonoxid may give rise to future ideas in the field of stem cell-loaded implants.
Effect of cold-setting calcium- and magnesium-matrices on protein expression in osteoblastic cells

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Introduction: Bone loss due to accidents or tissue diseases requires replacement of the structure by artificial materials. Commonly used materials in non load bearing areas like the cranio-facial region are based on calcium phosphate chemistry, e.g. sintered hydroxyapatite or β-tricalcium phosphate ceramics or self setting calcium phosphate cements. A novel material class for bone replacement are magnesium phosphate based cements, where the setting products newberyite and struvite can be resorbed by the host under physiological conditions and replaced by bone. Aim of this study is the biological testing of different calcium- and magnesium phosphate based cement compositions.

Material and Methods: Composition and cement products are shown in table 1. Cylindrical specimens of 15.5 mm in diameter and 3 mm in height were formed by mixing powder and liquid in a silicon mould and samples were sterilised after setting by either autoclaving or incubation in 70% ethanol. Phase composition was been determined by means of X-ray diffraction analysis and compressive strength was measured on wet samples with an aspect ratio of 2:1 using a statitical test equipment Zwick 1440 (Zwick-Roell, Ulm, Germany). Cell growth and cell activity of Osteoblastic cells (MG 63) on the different surfaces were determined after 2, 4, 7 and 10 days and the expression of some bone marker proteins was analysed by western blotting.

Results: Magnesium phosphate cements showed reduced compressive strength compared to hydroxyapatite. Cell growth and protein expression on the different cement matrices were compared to the already clinically used hydroxyapatite (CDHA). Cell activity normalized to cell number revealed higher activity of the osteoblasts on Newberyite and Struvite when compared to CDHA. The expression of osteoblastic marker proteins was highest on Brushite scaffolds.

Discussion: The presented cement matrices based on calcium- and magnesium phosphate show better biocompatibility than conventional hydroxyapatite cements. Advantageous of these materials is the good resorbability in vivo due to a higher chemical solubility. While Brushite and Newberyite are setting under acidic conditions, formation of Struvite occurs under physiological pH like hydroxyapatite cements providing the possibility of additional modifications with proteins or other active components.

<table>
<thead>
<tr>
<th>Cement powder</th>
<th>liquid phase</th>
<th>setting product</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Ca₃(PO₄)₂</td>
<td>2,5% Na₂HPO₄</td>
<td>Ca₆(PO₄)₃HPO₄(OH)₂ (calcium deficient Hydroxyapatite, CDHA)</td>
</tr>
<tr>
<td>-Ca₃(PO₄)₂ / Ca(H₂PO₄)₂</td>
<td>H₂O</td>
<td>CaHPO₄ x 2H₂O (Brushite)</td>
</tr>
<tr>
<td>Mg₃(PO₄)₂ / MgHPO₄ x H₂O</td>
<td>20% H₃PO₄</td>
<td>MgHPO₄ x 3H₂O (Newberyite)</td>
</tr>
<tr>
<td>Mg₃(PO₄)₂ / MgNH₄PO₄</td>
<td>3.5M (NH₄)₂HPO₄</td>
<td>MgNH₄PO₄ (Struvite)</td>
</tr>
</tbody>
</table>

Table 1: Composition of the cement matrices
Virtual surface analysis of the membrane morphology of infected vital macrophages

Michael Granseier¹, Heike Grassmé², Erich Gulbins², Hans-Gerd Lipinski¹

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Introduction: During the infection of mammalian macrophages with pathogene bacteria a special deformation of the cell membrane occurs. In order to understand the whole process of cell infection this kind of membrane deformation plays an important role in micro-biologic studies [1,2]. By the use of modern 3D laser scanning microscopy it is possible to generate digital image stacks of cells which were previously stained with fluorescent dyes. Using computerized adapted 3D surface reconstruction algorithms methods these image stacks were assembled to three dimensional cell models which allows a detailed interactive analysis of the membrane surface and its distortion.

Material and Methods: Microscopic slices of mammal macrophages were available to generate the threedimensional reconstructions of the cell surface. Laser scanning microscopy was performed on a LEICA DMIRE 2, equipped with an × 100 oil objective. The planar extension of all probations was 2 150 150 m µ × . Its thickness ranged from m µ 094 , 4 up to m µ 538 , 9 . During image acquisition from each cell probation six slices were registered from top to bottom whereas the fluorescence dyes were stimulated by laser light. Each image had a size of 512 512× pixel which relates to a pixel size of 2 293 , 0 293 , 0 m µ × .

Results: After removing distracting objects and pixels from the images a linear interpolation of intermediate images was performed to increase the number of slices from six up to 21. For reconstructing the three dimensional images of the cells from the interpolated images we used two well known reconstruction algorithms, the “Marching Cube” and “Texture Mapping”. Calculations were performed using the Insight Toolkit (ITK) and the Visualization Toolkit (VTK) implemented with Microsoft® Visual Studio .NET 2003TM. During bacterial infection of the macrophages a characteristic deformation of the membrane underneath and around the bacteria could be observed. First we observed a dent under the bacteria. Second, there was also an elevation of the cell surface membrane at the edges of the bacterial aggregation. These topological membrane deformations could be recognized at the three-dimensional models due to surface reconstruction or virtual light reflections.

Image A in figure 1 shows the three-dimensional reconstruction of a macrophage performed with the “Marching Cube” algorithm. With image B in figure 1 another macrophage is mapped reconstructed with the “Texture Mapping” algorithm.

Conclusion: By means of the two spatial reconstruction algorithms called “Marching Cube” and “Texture Mapping” it was possible to visualize the topography of the cell surface during an infection process. Especially in the membrane region of bacterial penetration a characteristic deformation of the membrane along the bacterial aggregation could be
observed. This special topological shape points out an important reason why the bacteria are able to diffuse into the macrophages. The "Marching Cube" method is well suitable for reproducing the surface structure of the cell whereas "Texture Mapping" produces a realistic view of the different colour-intensities and luminance of the cell membrane.

References:

**Introduction**: Silver nanoparticles (Ag-NPs) are increasingly used in different areas, such as electronic, clothing industry or food. In particular, Ag-NPs has been used in a range of medical settings such as silver coated catheters due to well-known slow-release antiseptic properties. Released silver ions exert antimicrobial properties after binding to microbial DNA, cell wall components or sulfhydryl groups of the metabolic enzymes. Subsequently, the bacterial replication and different metabolic pathways are interrupted. Despite the widespread use of nanosilver there is a serious lack of information concerning the biological activities of nanosilver on human tissue cells.

**Materials and Methods**: The study evaluated the biological influence of spherical Ag-NPs (diameter about 100 nm) on cellular functions (viability, cytokine release and chemotaxis) of human mesenchymal stem cells (hMSCs). Silver nanoparticles were prepared by the polylol process, i.e. by the reduction of silver nitrate with ethylene glycol. The silver nanoparticles were colloidally stabilized by the polymer polyvinylpyrrolidone (PVP). hMSCs were treated with or without PVP-functionalized spherical Ag-NPs (concentrations 50 ng mL\(^{-1}\)– 50 µg mL\(^{-1}\)) up to 7 days under cell culture conditions. Control experiments with silver ions (diluted silver acetate) were performed additionally to separate particle and ion effects. Cell viability was determined after calcein-AM staining, chemotaxis of hMSC was quantitated after membrane transmigration and subsequent cell staining. Cell proliferation was analyzed using the AlamarBlue-assay. Cytokines were determined by ELISA technique and Ag-NPs agglomeration was analyzed by the Zetasizer Nano ZS (Malvern, Germany).

**Results and Discussion**: Cytotoxic cell reactions occurred at Ag-NPs levels above 5 µg mL\(^{-1}\). Chemotaxis and cell proliferation of hMSC decreased with increasing nanosilver concentrations. In the presence of Ag-NPs/Ag-ions different effects on cytokine release from hMSCs were observed. At sublethal concentrations of Ag-NPs (2.5 µg mL\(^{-1}\)) the release of IL-8 was significantly increased, in contrast, the levels of IL-6 and VEGF were decreased compared to the control. The synthesis of IL-11 was not affected in the presence of different Ag-NPs-concentrations. The size of Ag-NPs agglomerates increased in media with a high electrolyte content, i.e. RPMI. Complexation with proteins (e.g. albumin) in the media stabilized the silver nanoparticles against agglomeration. In summary, the results show that Ag-NPs exert cytotoxic effects on hMSCs at high concentrations but also induced cell activation (as analyzed by IL-8 release) at sublethal concentrations of Ag-NPs.

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Polyurethane Biomaterials for Sorption the Phenols from the Water Solutions

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Remove the phenols from the water solutions especially from the waste waters is one of the main problems of modern waste water treatment. This research concerns obtention and exploration of polyurethane sorbents using microbe polysuccaride xanthan as hydroxyl-containing component [1]. A new enviromental friendly technology of polyurethane obtaintion is proposed. It is possible not only due to the microbe polysaccharide using but also due to applying blocked polyisocyanates [2]. This technology supplies formation of polyurethane networks which have sorptive abilities. Method of the electrone spectroscopy allows to evaluate sorptive ability of the obtained materials and also to observe the correlation of the hydroxyl groups substituted in polysaccharide with the phenol sorbed from the water solution. There two stages of the process. The first one concerns the water concentration and the second one is the stage of phenol sorption.

It is also important to substitute toxic isocyanates with their environmentally safe equivalent. Besides, the materials are thermoresistible and resistible in aggressive mediums and are also able to sorb transition metal ions from water solutions. These properties could render possible application the obtained films as sorbents for wastewater treatment.

References


UV-specters of the:
initial phenol water solution $10^{-4}$M;
solution after contact with sorbent during 24 hours;
solution after contact with sorbent during 72 hours
Observation of hydrothermally induced phase transformation of yttria-stabilized ZrO$_2$-ceramics for dental applications.

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ZrO$_2$-ceramics are used if high hardness, high wear resistance, high corrosion resistance or high-temperature stability is requested. Because of its biocompatibility, zirconia is applied in biomedical engineering, especially in dentistry as a material for dental implants, endodontic posts, brackets and, more recently, for inlays, crowns and bridges. Zirconia crystallizes in three phases: cubic, tetragonal and monoclinic. At room temperature, pure zirconia is monoclinic and transforms at 1170 °C to tetragonal and at 2370 °C to cubic. However, the application of zirconia in a temperature range from 120 °C to 300 °C in humid air/water vapor is limited by low-temperature degradation (LTD), which means a premature transformation of tetragonal phase to monoclinic phase (t→m) in connection with a volume increase leading to crack formation and deterioration of mechanical properties. In order to retain the tetragonal phase at room temperature and to prevent LTD from taking place, ZrO$_2$ must be doped with stabilizers like Y$_2$O$_3$, CeO$_2$, MgO, CaO, etc. [1-4].

In this work phase transformation of Tetragonal Zirconia Polycrystals (TZP) to monoclinic phase induced by hydrothermal ageing at 80 °C in water was investigated by means of X-ray diffraction (XRD). Three materials of 3 mol% yttria stabilized TZP exhibiting different Al$_2$O$_3$ contents were aged for 16, 32, 48, and 64 days. Two of these materials - Lava (3M ESPE) and Z700K (BCE Special Ceramics GmbH) - were doped with additional 0.25 mass% Al$_2$O$_3$, the third one - Ziraldent® (Metoxit AG) - with additional 20 mass% Al$_2$O$_3$. All samples existed as discs (Ø 14 mm, h = 1.2 mm). The discs made from Z700K and Ziraldent® were used “as fired” while the discs made from Lava were polished up to 1 µm diamond paste. XRD of Lava, Z700K and Ziraldent® was accomplished before ageing and after each ageing increment of 16 days. XRD profiles were measured using an X-ray powder diffractometer (Bruker AXS D4 Endeavor) with monochromatic CuK$_{α1,2}$ radiation, under 40 kV and 40 mA. Scans were performed from 15° – 110° (2θ) at a step size of 0.03° with a measuring time of 4 s per step. All phases of zirconia were determined by Rietveld refinement using fundamental parameters with the DIFFRACplus TOPAS 3.0 software (Bruker). Starting lattice parameters were taken from literature [4-6].

After 64 days of ageing XRD-analysis gave different amounts of monoclinic phase. While Z700K exhibits the highest content of monoclinic phase up to 30 mass% Ziraldent® showed only 15 mass% and Lava the minimal transformation of about 10 mass%. Regarding these results it can be assumed that a higher content of alumina in yttria-stabilized ZrO$_2$ ceramics retards the tetragonal to monoclinic transformation. The examination of Lava samples shows that also for yttria-stabilized zirconia with a small content of alumina (0.25 mass%) the tetragonal to monoclinic phase transformation can be retarded drastically if the surface of the sample is polished.

In addition to XRD analysis biaxial bending tests were performed according to DIN EN ISO 6872. Mechanical testing, however, did not reveal a significant change of mechanical properties after 64 days of hydrothermally ageing. This means that after short periods of ageing tetragonal-to-monoclinic transformation occurs on the surface only and has no significant influence on bulk properties.

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Influence of chitosan additions on osteogenic properties of calcium phosphate coating obtained by means of micro arc oxidation

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The main problem faced by orthopaedic implant manufacturers is creation of bioactive and osteogenous surface. For a long time they use for this aims calcium phosphate coatings. However, search of implant surface modification methods is continuing. The present paper is devoted to study of osteogenous properties of calcium phosphate coating applied with micro arc oxidation method which has been modified by means of chitosan addition.

One used Ti6Al4V disks with 9 mm diameter and 2 mm thickness as substrate for coating application. Before coating application the disks were purified by means of chemical defatting and treated with ultrasound in distilled water. To make the calcium phosphate – chitosan composite the 5 % solution of chitosan (85% deacetilation degree, CJSC “Bioprogress”, Moscow) in 1 % lactic acid has been prepared. Solute polymer was added to the Ca-P electrolyte. The electrolytes with chitosan concentration of 0.02, 0.125 and 0.5 g/l have been prepared. The coating was applied by micro arc oxidation method at temperature 28 – 30 °C in 15 min. with current density 0.1 – 0.4 mA/cm² (in dependence on chitosan concentration). After applying of coatings the samples were rinsed in distilled water and dried in dry-air sterilizer at temperature 60-70 °C in 10 -15 min.

Micro- and mesorelief of coating surfaces has been studied by means of a Philips SEM 515 scanning electron microscope. As obtained results show all coatings are deposited uniformly on the whole surface of the titanium disks. Calcium phosphate (Ca-P) and calcium phosphate –chitosan (CaP-C) coatings were formed from Ca-P globules with diameter ranged from 80 nm to 150 µm. Ca-P coating consisted of spherolite-like crystals forming mesorelief of coating surface with pore size ranged from 5 to 250 µm, with surface porosity about 60%. Isolated pores were localized in spherolites, through pores were located on the spherolite borders. CaP-C coatings were formed from spherolites, too, but surface relief was more smoothed due to coverage of calcium phosphate globules with chitosan. Their porosity was 35% and average pore size about 15 µm. On the CaP-C coating majority of polymer aggregates both are concentrated around Ca-P globules, and cover them. Content of chitosan aggregates in coating is increasing with its concentration in solution. Sometimes binding of Ca-P and chitosan with formation of grid structure takes place.

The osteogenous properties of implants were studied in experiment by means of ectopic bone formation method using the BALB/c line mice. The implants with column of syngenous bone marrow, which has been preliminary applied in aseptic conditions, were inserted in animals under ether narcosis. After 1.5 months the implants were revealed. The histological analysis has been carried out by standard method of light microscopy of thin section of tissue platelets, grown on the implants and painted with hematoxilin-eosin. Results were varied from 75 % formation of tissue platelets and 50 % formation of bone tissue in samples without chitosan up to 100 % formation of bone tissue with one marrow at chitosan concentration of 0,5 g/l. Thus addition of chitosan to calcium phosphate improves its osteogenic properties and can be recommended for clinical use.
Modification of bone tissue engineering in vivo by RGD peptides deposed on nanostructured calcium phosphate coating

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Arginine-glycine-aspartic acid (RGD) recognition sequences in adhesive molecules (fibronectin etc.) are one of the principal cell adhesion participants which are high-affinity ligands for cell integrine receptors on cells including bone marrow stromal progenitor and stem cells. Besides, RGD peptides promote epithelium cells adhesion with subsequent angiogenesis. Osteogenous properties of hydroxilapatite and tribasic calcium phosphate (CP), which are the base of mineral bone matrix, are obviously proved per ectopic osteogenesis phenomenon, when bone tissue is generated on the surface of CP materials. In this research we have examined regulating effect of RGD peptides on bone tissue formation from bone marrow cells in vivo.

Rough (Ra=3-6 µm) and smooth (Ra=1,5-2 µm) CP coatings have been deposited on titanium discs by anodic spark technology using synthetic and biological hydroxyapatite (HAP) nanoparticles (20-40 nm). Commercially available RGD (Bachem AG) in powder form was deposited on the implant CP coatings by thermal evaporation technique using vacuum system (base pressure 10⁻⁶ Torr) equipped with variable temperature heater, which was maintained at approximately 180°C during the deposition process [3]. After the process, the system was cooled down gradually to room temperature by simply turning off the power supply of the heater. Optically homogeneous, 1 µm thick film of RGD was observed over all the substrate exposed area. Studies in situ were implemented by the method of ectopic bone formation when treated samples with bone marrow cells were subcutaneously implanted in mice Balb/c for 45 days without additional injection of growth factors. Thin transverse tissue plates sections were prepared and stained with hematoxylin-eosin. Areas of bone (osteoblasts function), marrow (hemopoiesis inducing microenvironment function) and marrow lacunae (osteoclasts function) were determined.

Application of rough CP coatings for tissue bioengineering increases in 1,5-2 times the bone tissue area, formed by osteoblasts, in comparison with smooth ones. The roughness probably facilitates adhesion and differentiation of multipotential mesenchymal stromal cells in osteogenous direction. CP surface modification by RGD tripeptides has significantly decreased bone marrow adhesion. It was fixed due to decrease (28-75 %) of average area of cross-sections of grown tissue plates independently on type of coating and kind of HAP. The maximal index decrease was noted in case of rough CP coatings. Histological composition of cross sections showed that relative bone tissue area was in reciprocal dependence on marrow and marrow lacunae area. At the same time the absolute (mm²) figures show that treatment of CP coatings with RGD tripeptides suppresses bone tissue, marrow and marrow lacunae formation (67 % of cases) or, on the contrary, stimulates hemopoiesis (33 % of cases). RGD tripeptides modulate functional activity and differentiation of stromal and hemopoietic marrow cells. Nevertheless, they inhibit adhesion of marrow stem cells and impair bone tissue remodeling. This fact may restrict their application for bone tissue engineering in vivo on rough CP coatings.

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Perfluorocarbon-filled poly(lactide-co-glycolide) capsules as potential artificial oxygen carriers for blood substitutes

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Poly(lactide-co-glycolide) (PLG) copolymers are one of the most innovative biodegradable materials being intensively investigated for an effective drug delivery system of hydrophobic, low and high molecular weight compounds. The synthesis of perfluorocarbon-filled micro- and nanocapsules with PLG as wall material has been recently suggested for ultrasonic imaging. In the present study encapsulation of perfluorodecalin by PLG was applied to gain a potential artificial oxygen carrier for blood substitutes. The morphology of the microcapsules and their in vitro clearance by calcine labeled macrophages and endothelial cells, respectively, was studied by confocal laser scanning microscopy using Nile red as second fluorescent marker to stain the capsule walls. The mechanical stability and the wall flexibility of the nanocapsules were examined by atomic force microscopy. It was found that the preparation in fact led to nanocapsules with a mechanical stability comparable to red blood cells. The permeability of the capsule walls for dissolved molecular oxygen was detected by nuclear magnetic resonance spectrometry. It was found that the positions of some F-19 NMR resonances of the liquid content perfluorodecalin as well as their relaxation times strongly depended on the actual molecular oxygen concentration. Simple kinetic studies on this basis are presently restricted to a temporal resolution of about 30 seconds, but so far indicate that molecular oxygen penetrates rapidly the capsule walls. In order to decrease a putative “opsonisation” process in biological environments, the capability of the capsule wall to adsorb immunoglobulins and other plasma proteins was lowered by coating with a block copolymer of ethylene oxide-propylene oxide (tetronic 908, BASF), but other surfactants are presently under investigation. However, our data clearly demonstrated that a suspension of encapsulated perfluorocarbon rather than commonly used perfluorocarbon emulsions can be used as artificial oxygen carriers.
Flexible fibrous biomaterials modified by hydroxyapatite

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**Introduction:** Biomaterials based on synthetic hydroxyapatite (HA) Ca_{10}(PO_{4})_{6}(OH)_{2} are well-known for replacing damaged bones of living systems and for functioning in an intimate contact with tissues [1–3]. The main goal of our work is an elaboration of biomaterials based on flexible fibrous substrates (cellulose, carbon) modified by HA promising for various medical applications.

**Materials and Methods:** The HA gel was prepared by the interaction between CaCl_{2} and (NH_{4})_{2}HPO_{4} aqueous solutions at Ca/P ratio 1.67 and pH 10–11 by known method [4, 5]. The HA powder was prepared by drying HA gel at 60°C up to fixed-mass, turn into the powder (particle size ≤3 μm).

Cellulose was used in initial form and after phosphorylation with ion-exchange capacity of 0.8; 1.7; 3.4 mg eq./g. Cellulose fibers were modified by 3–7% HA gel and 3–5% polyvinyl alcohol (PVA) solution with 3–7 mas.% HA powder and 5% glycerol (plasticizer) during 5 min to 2 h.

Carbon fibers (high- “LT-2-22/45” and low- “LT-5/45” temperature) and felts “Karbopone” were modified by

i) 3.3% HA gel without and together with 5% PVA solution during 5–60 min;

ii) centrifugation in 3.2% HA gel at 3000 rpm, 10 min;

iii) electrochemical deposition of calcium phosphate coatings in 0.624/0.374 M (NH_{4})_{2}HPO_{4} aqueous solutions at Ca/P ratio 1.67 and pH 10–11 by known method [4, 5].

The mass of electrochemically deposited calcium phosphate coatings on carbon fibrous substrates increases from 28 to 70 mg and on carbon felts – from 77 to 306 mg in direct proportion to current density of 5–20 mA/cm^{2} and deposition time of 10–20 min. SEM images are show that lamellar calcium phosphate crystals are formed in the bulk (inter-fiber space) of carbon substrates.

With the use by XRD and FTIR techniques we found that the calcium phosphate coatings on carbon substrates consist of calcium hydrogen phosphate dihydrate (brushite) CaHPO_{4}·2H_{2}O, α-tricalcium phosphate α-Ca_{3}(PO_{4})_{2}, octacalcium phosphate Ca_{10}H_{2}(PO_{4})_{6}·SH_{2}O mixture.

At the present time the biomaterials based on flexible fibrous substrates modified by HA are testing (pre-clinical trials) for the preparation of ophthalmologic, maxillofacial implants (carbon / HA) and proctologic implants (cellulose / HA) as alternative of porous ceramics.

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rh-BMP-2/Algisorbe/Poly-(D,L)-Lactide-membranes for Bone Tissue Engineering

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Introduction: Polylactides are one of the most important biodegradable polymers for use in medicine. At present they only play a minor role as a bone replacement material. An osteoinductive composite of Algisorbe/PDLLA with rh-BMP-2 would probably expand its role as a drug device in bone therapy. Here we show that the rh-BMP-2 loading capacity in Algisorbe/PDLLA-membranes was up to 0.9 mg rh-BMP-2/g Algisorbe with a release half-life in the range of 23 d as determined with $^{125}$I-rh-BMP-2.

Materials and Methods: Recombinant human bone morphogenic protein 2 (rh-BMP-2) was prepared in E. coli [1]. Radioactive rh-BMP-2 was prepared by labeling with $^{125}$I according to Chloramine-T method [1]. PDLLA was purchased from Boehringer-Ingelheim and solved in an organic solvent. The PDLLA solution was effused in forms and dried under standard pressure. On the semi-dry PDLLA-membranes the Algisorbe (a gift from AlgOss Biotechnologies GmbH) was fused respectively. After that the membranes were dried for 12 h and cut into little strips. The composite-strips were incubated in $^{125}$I-rh-BMP-2 solution on a rotary wheel at room temperature for 13 h, washed three times and at finally lyophilized.

For measuring the release kinetics of $^{125}$I-rh-BMP-2, the composite-strips were incubated in PBS-buffer (pH 7.4) with multiple buffer changes of 1.5 ml as described previously [1] on a rotary wheel at room temperature for 14 days. At indicated times, the composite-strips were taken out of the buffer and washed two times in fresh PBS-buffer for measurement in a $\gamma$-counter. The calculated half-lives were corrected for the spontaneous decay of $^{125}$I ($t_{1/2} = 59$ d). The biological activity of rh-BMP-2 was tested with the cell line MC3T3-E1 by the induction of the novo synthesis of alkaline phosphatase [1].

Results: As shown in Figure 1, incubation of the $^{125}$I-rh-BMP-2-composite-strips in PBS-buffer (pH 7.4) for 14 days, results in a two-phase-exponential release of $^{125}$I-rhBMP.

Figure 1
Two-Phase exponential release of $^{125}$I-rh-BMP-2. The decay curves were non-linearly fitted to two phase exponential equation[1].

The release-kinetic of rh-BMP-2 was a first-order-reaction with two phases. The initial release burst occurred in the first phase from day 1 - 2 and a slower releasing rate followed in the second phase from day 3 - 23. According to this releasing behavior, two half-lives can be determined. Initial phase: 0.17 d ($k_1 \sim 5.0 \times 10^{-5}$ [s$^{-1}$]), second phase: 23 d ($k_2 \sim 3.0 \times 10^{-7}$ [s$^{-1}$]). In the day 1-2 where 13 % of the doped rh-BMP-2 was released, followed by a slow phase, between day 3-20, where 23 % rh-BMP-2 was released. It could be shown, by induction of alkaline phosphatase and the determined $K_D$-value, that rh-BMP-2-composite-strips were biological active.

Conclusions
I) The rh-BMP-2 loaded membranes and released rh-BMP-2 shows biological activity.
II) The assembly of the composite-membranes have no significant effect on the biological activity and released rh-BMP-2.

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ATOMIC FORCE MICROSCOPY FOR PREDICTION THE VASCULAR TISSUE REACTION
(Experimental study)

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Keywords: Restenosis; Biocompatibility, AFM; adhesion force; tip modification

Introduction: The main cause of in-stent restenosis developments is the body reaction to the exogenous material. The level of restenosis developments is closely connected with immune status of experimental animals. Atomic force microscopy (AFM) technique is the prospective method for compatibility testing the stent’s material to the living body before operation [1]. This work discusses the correlation between neointima growth and the adhesion forces that were measured by AFM method with tip modified by recipient’s antibody [2-3]

Mate Materials and Methods: The study was carried out on experimental animals, 28 rabbits, male, weight 2.5-3 kg. The week before operation the plates with 4 coatings corresponded to the stents coatings were implanted into the animals’ muscle on the back. On the 7th day after plates’ implantation the samples of animals’ blood were collected and the summary IgG were separated by routine method. The IgG were applied on the AFM tip and the adhesion forces were determined by atomic-force microscope Dimension 3000 NanoScope IIIa (patent application # a2007 09030). At the same day stents with corresponded coatings were implanted into rabbit’s abdominal aorta. In 8 weeks after stents insertion the animals were sacrificed. The stented sites of aorta were collected for the further histological study and morphometry. The level of neointima growth was compare with AFM data.

Results: The minimum vessel thickness, 212 ± 19 mkm, was corresponded to the mean of adhesion force about 16-22 nN. In the case when the adhesion forces were over 35 nN the thickness of vessel wall increased in times and became 415±126 μm.

Conclusion: The adhesion forces with which stents material hold modified with IgG AFM tip reflects the body reaction and could predict the restenosis developments.

References:

Early Clinical and Radiographic Results of Porous Tantalum Primary Hip and Knee Prostheses

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Introduction: The advent of highly porous metals has led to the development of new primary porous tantalum hip and knee prostheses. Although primary total joint replacements have achieved high levels of success, the traditional metals they are fabricated from maintain certain intrinsic limitations. The goal of this poster is to introduce the latest porous tantalum primary hip and knee prostheses and report on their early clinical and radiographic results.

Methods: A retrospective review of primary modular porous tantalum hip and knee components was performed. In reviewing consecutive cases from 2006 to 2008, we found 31 cases of modular acetabular implants, 84 primary femoral hip prostheses, and 24 modular tantalum tibial trays. Patient demographic and clinical data were compiled for each of these implants. Follow-up clinical and radiographic examinations were obtained at six weeks, six months and yearly thereafter for all available cases. Standard hip and knee scores were evaluated pre- and post-operatively and radiographs were evaluated for evidence of wear, osteolysis and loosening.

Results: The average length of follow-up for primary total hip arthroplasty was 18 months (range 6 to 24 months) and 9.3 months for total knee arthroplasty (range 6 to 12 months). The average age of patients undergoing hip surgery was 64.6 years old (range, 26 to 95) and for knee surgery 63 years old (range, 39 to 81). Three patients passed away at early follow-up, unrelated to their total joint arthroplasty. All remaining patients were available for clinical and radiographic evaluation at latest follow-up. Two patients with a porous tantalum tibia experienced early radiographic subsidence (2 mm and 4 mm) with subsequent stabilization. There were no revision surgeries in this group of patients. In the 31 cases of modular acetabular components there was one deep infection and no other complications noted. For patients with a porous tantalum primary hip prosthesis there were three traumatic hip dislocations all treated successfully with closed reduction (in all three cases patients had a prior open reduction and internal fixation of the affected acetabulum). There were no cases of septic or aseptic loosening in this group of patients.

Conclusions: Early clinical and radiographic results are favorable using porous tantalum primary hip and knee prostheses. These early results suggest this new porous metal is a viable option for biologic fixation in primary hip and knee arthroplasty. Long-term data/follow-up is necessary to validate our results and this early reported success.
A virtual 3D scene containing intercellular connections of vital bone cells

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Introduction: Laser scanning microscopes allow for creating three dimensional scenes of vital cells obtained from cell culture. A three dimensional image of bone cell scans shows a typical fibre network connecting the bone cells. Some of these fibres are connecting two cells, since other fibres are incomplete or cut. We developed a computer program which allows us to create a virtual scene of bone cells and the fibres to study some geometrical parameters of both cell bodies and connecting fibres.

Methods: We used an image stack containing 44 images of bone cells (new born rat) generated by a standard laser scanning microscope. The images consisted of 1344 x 1025 pixels each (8 Bit gray tone value). The raw image data were smoothed by diffusion filtering. The 3D-Scens were created by a modified marching cube algorithm which computed surface data of the cellular object.

Results: 3D-reconstructions based on image raw data mostly show incomplete connections between two cells (cf. Figure 1A). We virtually cut all connection fibres and removed them from the image data. Additionally, we smoothed the remaining cell body data and created a model scene of the cell bodies (cf. Fig. 1B). Next, the cells were randomly connected by a defined number of model fibres. Fig. 1C shows a scene of two cells connected with 2 fibres while Fig. 1D contains a model scene with 7 fibres. Thickness and main direction of the model fibres were computed from geometrical fibre data obtained from the raw data set.

From our model calculations some geometrical cell parameter derived. The mean fibre diameter was 1.96 μm (n=36; range: 0.78 – 4.10 μm) while extension ranged from 14 μm up to 17 μm (mean value: 15.8 μm). The cell body volume ranged from 500 μm³ up to 700 μm³ with a mean of 550 μm³ (n=12). The volume of one model fibre was approximately 50 μm³. Hence, cell volume equals approximately 10 times of a fibre value.

Conclusion: We developed a noise reducing 3D-reconstruction algorithm which allows us to compute a virtual scene of vital bone cells using laser scanning microscopy data. Main objects of the scene (cell body and connecting fibres) could be separately generated and randomly connected. From the scene some important geometrical data could be obtained to estimate both cell body volume and fibre volume.

Fig. 1: 3D-Reconstructed scene of two living bone cells obtained from Laser scanning microscopy. A: original bone cell scene obtained from raw data. B: Model scene created by smoothed and low pass filtered image data using a modified Marching cube algorithm. C: Two cell models randomly connected by two fibres. D: Two cell models randomly connected by seven fibres.
Introduction: Titanium is widely used as biomaterial for dental implants. Hereby the surface texture is of great importance. In the last decade the so called SLA (sandblasted and acid etched) surface was developed which shows promising properties [1,2]. Moreover, it is assumed that the bioadhesive and osteophilic properties of a surface increases with increasing hydrophilicity [3]. The aim of this work is to prepare a defined SLA surface on titanium which should be hydrophilized by the chromosulfuric acid method developed in our group several years ago [4].

Materials and Methods: In order to optimize the etching process in respect of surface texture and hydrophilicity smooth, machined titanium mini-plates (grade 4, size 14 x 14 x 1.6 mm, Camlog, Switzerland) were selected as model material for implants. The machined titanium was blasted with corundum obtaining the so called sandblasted surface (standard pressure (= SBI) or reduced pressure (= SBII)). The micro-structured so called SLA-M surface was generated by etching the sandblasted titanium mini-plates in sulfuric acid (SA) at elevated temperature. For kinetic investigations reaction times between 30 s and 600 s were checked. The nano-structured so called CSA-M surface was prepared by treatment of SLA titanium by chromosulfuric acid (CSA) at 210 °C for 30 min [4]. The samples were characterized by measurement of the dynamic contact angle in water (Wilhelmy plate method, Tensiometer DCAT 11, Dataphysics), scanning electron microscopy (SEM) and energy dispersive X-ray analyses (EDX).

Results: SLA-M Surface. The sulfuric acid treatment of sandblasted titanium at elevated temperature leads to highly hydrophilic surfaces. If the samples are transferred to ultra pure water after etching and not exposed to air, in general dynamic advancing angles below 10° are measured (table 1). The samples loose their hydrophilic properties, however, after storing in air for hours. After a short reaction time of less than 2 minutes a characteristic comb-shaped microstructure is developed by sulfuric acid (fig. 1A). This structure disappears after longer treatment than 5 min. Furthermore, the corundum content is considerably reduced for the SLA-M surface compared to the sandblasted surface (table 2). In the case of using reduced pressure for the sandblasting process (SBII) the Al/Ti ratio detected by EDX decreases from 21-26 Atom% to 0-2.5 Atom%.

Table 1: Statistics of the Contact Angle Measurements on SLA-M and CSA-M Surfaces

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>$\bar{\theta}$ (adv) ± SD</th>
<th>$\bar{\theta}$ (rec) ± SD</th>
<th>Both Angles &lt; 10°</th>
<th>Both Angles = 0°</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBI + SA</td>
<td>56</td>
<td>0.4 ± 1.5°</td>
<td>0.0 ± 0.0°</td>
<td>100%</td>
<td>93%</td>
</tr>
<tr>
<td>SBI + SA + CSA</td>
<td>26</td>
<td>1.3 ± 4.6°</td>
<td>0.0 ± 0.0°</td>
<td>96%</td>
<td>88%</td>
</tr>
<tr>
<td>SBII + SA</td>
<td>22</td>
<td>6.5 ± 13.9°</td>
<td>1.4 ± 4.1°</td>
<td>77%</td>
<td>73%</td>
</tr>
<tr>
<td>SBII + SA + CSA</td>
<td>24</td>
<td>5.9 ± 12.2°</td>
<td>0.0 ± 0.0°</td>
<td>79%</td>
<td>75%</td>
</tr>
</tbody>
</table>
### Table 2: Statistics of the Aluminum and Chromium Content Measured by EDX on Sandblasted, SLA-M and CSA-M Surfaces

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Al/Ti ± SD Mean Atom%</th>
<th>Al/Ti Max Atom%</th>
<th>Cr/Ti ± SD Mean Atom%</th>
<th>Cr/Ti Max Atom%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBI</td>
<td>1</td>
<td>30.84</td>
<td>-</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>SBI + SA</td>
<td>18</td>
<td>3.03 ± 2.63</td>
<td>9.15</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>SBI + SA + CSA</td>
<td>12</td>
<td>0.92 ± 0.66</td>
<td>2.26</td>
<td>0.28 ± 0.32</td>
<td>0.92</td>
</tr>
<tr>
<td>SBII</td>
<td>6</td>
<td>23.12 ± 2.24</td>
<td>26.07</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>SBII + SA</td>
<td>12</td>
<td>1.37 ± 0.88</td>
<td>2.51</td>
<td>0.04 ± 0.08</td>
<td>0.23</td>
</tr>
<tr>
<td>SBII + SA + CSA</td>
<td>9</td>
<td>0.15 ± 0.21</td>
<td>0.53</td>
<td>0.04 ± 0.08</td>
<td>0.23</td>
</tr>
</tbody>
</table>

### CSA-M Surface
Similarly the treatment of the SLA-M surface by CSA at high temperatures (> 200 °C) leads to highly hydrophilic surfaces (table 1). The hydrophilicity of this CSA-M surface can be conserved in methanol for several years [4]. SEM pictures show that the SLA micro-structure is partially preserved and nano-sized spheroids appear additionally (fig. 1B). Furthermore, the CSA etching reduces the corundum content to almost zero and no significant amounts of chromium were incorporated according to EDX measurements (table 2).

### Conclusion and Prospects
A defined SLA surface can be prepared on titanium by etching with sulfuric acid at elevated temperature. CSA treatment of this SLA titanium leads to a highly hydrophilic surface devoid of contaminations with a defined nano-structure, whereby the SLA micro-structure is partially preserved. Interestingly the freshly prepared SLA titanium mini-plates already show highly hydrophilic behavior. Further experiments should show, if it is possible to conserve the hydrophilicity of the SLA-M surface. Further on, it is necessary to transfer the results from the mini-plates to implants. In order to investigate the bioactivity and osteophilicity of the SLA-M- as well as the CSA-M surface in vitro and in vivo experiments are required.

### References


Biological activity of ultrahydrophilic and hydrophobic immobilized rhBMP-2 on a titanium sand blasted and acid etched surface

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Introduction: Our focus is to immobilized recombinant human bone morphogenic protein 2 (rhBMP-2) on chemically treated titanium surfaces. The rhBMP-2 on titanium implant device leads to enhanced bone growth and accelerated osseointegration in vivo [1]. We could show that the bound protein has the advantage release from the metal surface with a half-lives of 90 to 140 days. The aim of these study was to quantify the immobilization of the rhBMP-2 on ultrahydrophilic and hydrophobic modified sand blasted and acid etched (SLA-M) titanium-surfaces by using different protein concentrations and measuring the resulting biological activity in vitro.

Materials and Methods: Sand blasted and acid etched (SLA-M) titanium plates were treated by chromosulfuric acid (CSA) leading to ultrahydrophilic ($\theta < 10^\circ$) surfaces [2]. In a second set of plates the CSA treating is followed by a chemical reaction with 5% 3-aminopropyltriethoxysilane (APS) solution in toluene to lead a hydrophobic ($\theta \sim 70-80^\circ$) metal surface [3]. The rhBMP-2 adsorption was investigated using different protein concentrations. To determine the immobilized binding we used $^{125}$I-rhBMP-2. The biological activity of rhBMP-2 was tested with the cell line MC3T3-E1 by the activation of the novo synthesis of alkaline phosphatase. The cells are seeded on the surfaces and allowed to grow to confluence. The alkaline phosphatase can be measured and quantified by epifluorescence (relative fluorescence units, FU) [3].

Fig. 1: Adsorption of rhBMP-2 on i) ultrahydrophilic ($\theta < 10^\circ$) and ii) hydrophobic ($\theta \sim 70-80^\circ$) titanium SLA-M-surfaces vs the initial rhBMP-2 concentration.

Results: Fig. 1 shows the adsorption of rhBMP-2 on (i) ultrahydrophilic ($\theta < 10^\circ$) and ii) hydrophobic ($\theta \sim 70-80^\circ$) modified SLA-M-surfaces as a function of the initial rhBMP-2 concentration (0.0025 - 0.1 mg/ml). The adsorption curves could be fitted to a
hyperbolic function. The ultrahydrophilic modified surface is able to bind up to 1640 ng/cm², the hydrophobic surface up to 560 µg/cm² rhBMP-2 (c_initial= 0.1 mg/ml). Ultrahydrophilic surface bind 3 fold more than rhBMP-2 as the hydrophobic surfaces.

Biological activity of bound rhBMP-2 was measured in cell culture by alkaline phosphatase activity (epifluorescence). The green color in the epifluorescence indicates the biological activity of the bound protein. For both surfaces a) ultrahydrophobic and b) hydrophobic a biological activity can be shown (Fig. 2). Compared to the hydrophobic surfaces the ultrahydrophobic (which binds more rhBMP-2) shows higher biological activity.

**Conclusion:** The ultrahydrophilic and hydrophobic modified sand blasted and acid etched (SLA-M) titanium surfaces are suitable for immobilization of rhBMP-2. We could show that the bound protein rhBMP-2 shows a good biological activity in vitro. These results are fundamental for in vivo experiments with these surfaces.

**References:**

Improved wear performance of ultra high molecular weight polyethylene coated with hydrogenated diamond like carbon

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INTRODUCTION: Ultra high molecular weight polyethylene (UHMWPE) is the most relevant bearing materials used in total hip (THR) and knee (TKR). However, UHMWPE has been the weak material of these prostheses because of wear-related problems and mechanical degradation after longterm post-irradiation oxidation. Long-term osteolysis as a consequence of the physiological reactions triggered by polyethylene wear particles. To address this concern, highly crosslinked polyethylenes were used, which exhibit highly improved wear resistance due to an elevated crosslink density, but paradoxically HXLPE also exhibit decreased wear debris size, which may have the potential to increase biological activity and, then, aggravate osteolytic reactions [1]. Hydrogenated diamond like carbon (DLCH) coatings have shown good performances regarding hardness, wear resistance, friction, chemical inertness and good biocompatibility, which make them a good option for implants in biomedical applications[2]. The hypothesis of this work focuses on DLCH coated UHMWPE to decrease the risk of osteolysis, reducing the particle formation. To accomplish these goals, pristine and HXLPE were coated with DLCH and its surface nanohardness and tribological behavior were analyzed.

MATERIAL AND METHODS: The raw material used was a compression-molded sheet of Gur 1050 polyethylene (Perplas Medical Ltd., Lancashire UK). Discs 3 mm thick and 20 mm in diameter were machined from the sheet and were gamma irradiated in air (I) at 100 kGy (Aragogamma, Barcelona, Spain). DLCH thin films were deposited on UHMWPE by PECVD in a RF capacitive coupled reactor with plate parallel electrodes. DLCH hard layers of 250 and 700 nm were deposited on pristine (V) and irradiated (I) substrates. Several techniques were use to characterize the final DLCH coatings. Hardness profiles of the DLCH coated and uncoated UHMWPE samples were carried out using a nanoindenter XP MTS system. A commercial pin-on-disc tribometer (CSM instruments; Peseux, Switzerland) allowed assessing wear resistance and monitoring constantly the coefficient of friction for all the materials against alumina at 37 °C and with bovine serum like lubricant. Creep behavior of the UHMWPE was considered. Hardness profiles of the DLCH coated and uncoated UHMWPE were carried out using a nanoindenter XP MTS system.

RESULTS AND DISCUSSION: Hydrogenated diamond like carbon coated pristine and highly crosslinked UHMWPE samples present improved wear performances than the uncoated materials. Table 1 gives the results of the wear test. Tribological analyses reveal that 700 nm thick DLCH coatings are efficient to enhance the wear resistance of UHMWPE, although the friction coefficients in that case are higher than in the uncoated samples. The influence of DLC coating is higher on the crosslinked polyethylenes compared to the virgin polyethylene. DLCH coatings show good adhesion to the substrates as confirmed by nano-hardness analysis and surface morphology by ESEM and confocal microscopy. Therefore this type of coating is a good candidate to decrease the wear. These promising results must continue with new wear tests in simulators systems.

ACKNOWLEDGMENTS: We thank the Ministry of Science and Education of Spain (projects MAT2006-12603-C02-01 and CONSOLIDER-INGENIO CDS2008-0023 for financial support

REFERENCES:
1. Ries MD, Pruitt L. Clinical Orthopaedics and Related Research, 2005,440,149-156
### Table 1. Wear rate $K$ of uncoated and DLCH coated materials after 24 h sliding time

<table>
<thead>
<tr>
<th>Material</th>
<th>Wear rate $K \times 10^6$ (mm$^3$ / N m)</th>
<th>Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>2.38 ± 0.26</td>
<td>I</td>
</tr>
<tr>
<td>V250</td>
<td>2.03 ± 0.88</td>
<td>I250</td>
</tr>
<tr>
<td>V700</td>
<td>1.81 ± 0.13</td>
<td>I700</td>
</tr>
</tbody>
</table>
Dynamic mechanical properties of ultra high molecular weight polyethylene stabilized with vitamin E

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2Drexell University. Philadelphia USA

INTRODUCTION: Highly cross-linked polyethylenes by gamma or electron beam irradiation present a relevant improvement in wear resistance in load bearing and articulating components in total joint replacements. However the generation of free radicals during irradiation of the polymer in the presence of molecular oxygen produces an oxidation process may affect the material’s mechanical and physical properties. Update the stabilization step was carried out by subsequent annealing or remelting thermal process with partial success [1]. An alternative is the use of the α-tocopherol (vitamin E), which acts as a scavenger of residual free radicals generated by irradiation process, what allows replace the post-irradiation thermal process with the subsequent loss of mechanical properties involved, like toughness and fatigue resistance mainly. In order to design new components in total hip and knee replacements bases on this new and promising material, the global mechanical performance must be understand. Apartfrom the stiffness, toughness and fatigue of the polyethylene, whose dependence of the vitamin E is already known, the influence of the α-tocopherol on viscoelastic behavior and molecular dynamic must be established. This work deals with this last aspect in polyethylene’s where the vitamin E was introduced by two different methods, blending and diffusion, and also to the limitations of some techniques to detect the presence in the polyethylene of low vitamin E concentrations.

MATERIAL AND METHODS: The raw material used in this study was a compression-molded sheet of GUR 1050 (Perplas Medical Ltd., Lancashire UK) and GUR 1020 supplied by Meditech (Fort Wayne, USA) in several thin films. Vitamin E (α-tocopherol, Aldrich Chemicals) was introduced into UHMWPE by blending and by diffusion. The first method was used by Meditech to prepare films and the second in our laboratory using the standard protocol [2]. Dynamic mechanical measurements were carried out with a DMTA, model MKII from Polymer Laboratories in tensile mode. Vitamin E index were measured by FTIR spectroscopy, using a Pelkin-Elmer model 1600 equipment, and UV spectroscopy. Crystallinity content and melting temperatures were determined by DSC (Perkin Elmer).

RESULTS AND DISCUSSION: A linear correlation was obtained between VEI and wt % of vitamin E from the FTIR spectra at sample above 0.4 %. By UV spectroscopy a noticeable peak at 292 nm were observed in all the samples, including 0.07%, which increase with the vitamin E (Fig 1). The presence of vitamin E provokes a significant decreasing of the degree of crystallinity and in increasing of melting temperature. DMTA plots of storage modulus log E’(T) and loss modulus E’’(T) for all the samples show two
relaxations, one ranged at the highest
temperatures, \( \alpha \), related with the crystalline part and other at the lowest, \( \gamma \), associated to the amorphous phase. A third relaxation, \( \beta \), appears only in the GUR 1050 samples. Fig 2 shows the results for GUR 1050 with 0 (-), 0.7(-) and 9 %(-) of vitamin E introduced by diffusion. The presence of vitamin E introduces a new relaxation clearly noticeable at higher concentrations by a peak around -25 °C. Besides, the vitamin E modifies different aspects in the molecular dynamic of the matrix, but any plasticizer effect happens at relative high vitamin E concentrations. Activation energies were also calculated for all the relaxations.

ACKNOWLEDGMENTS: We thank the Ministry of Science and Education of Spain (projects MAT2006-12603-C02-01 and Consolider-Ingenio CDS2008-0023 for financial support

REFERENCES:

Untersuchung zeitaufgelöster hochaffiner Wechselwirkungen am Beispiel des Avidin-Biotin-Systems auf einer Sensoroberfläche

Analysis of time-dependent high affinity interchanges by the example of the avidine-biotin-system on a sensor surface.

M. Meissner

The measurement of binding kinetics of macromolecular substances (proteins, DNA) on plane solid surfaces in liquids is the principle of many evanescent wave and surface plasmon resonance biosensor systems. The analysis of macromolecules or very fast reactions, like immune reactions and DNA-hybridizations, meets the limits of these biosensor systems. The resulting constants are not reaction-limited but limited by mass-transport because molecules must move through a 5-10 µm thick Nernst diffusion boundary layer to the sensor surface.

By using a rheometer in connection with evanescent wave technology (Total Internal Reflection Fluorescence, TIRF) we succeeded in eliminating the Nernst diffusion boundary layer limitation by reducing this layer to nanodimensions with a thickness of 50-200 nm. Macromolecules can move through this layer without measurable transport limitation [1].

As an example for a biochemically specific interaction the avidin-biotin-system was studied. With 10^{15} M^{-1} it displays the highest binding constant in nature [2]. Therefore quartz glass plates was covalently bound biotin were prepared. Nonspecific binding areas on the glass surface were blocked with bovine albumin and the binding kinetics of FITC-labelled avidin to sensor surface were analyzed. The obtained kinetics could be fitted to a two phase exponential association and yielded two observable rate constants (k_{obs}). When the k_{obs} values were plotted versus the protein concentrations the corresponding sorption rate constants k_{+1} and k_{-1} could be obtained. Together with the above adsorption rate constants (k_{+1} and k_{+2}) an increase of the binding constants by one order of magnitude (K') can be calculated. From additional separate desorption experiments as shown in Fig. 2, the constants k_{-3} = \left(6.80 \pm 0.10 \right) \cdot 10^{-5} s^{-1} and k_{-4} = \left(1.31 \pm 0.02 \right) \cdot 10^{-5} s^{-1} indicative of further rearrangement reactions on the surface could be obtained. Together with the above adsorption rate constants (K_{+1} and K_{+2}) an increase of the binding constants by one order of magnitude (K') can be calculated.

![Fig. 1 Plot of observable rate constants k_{obs,1} vs. Protein concentration c to determine the sorption rate constants k_{+1} and k_{-1}. The sorption rate constants k_{+2} and k_{-2} results from plot k_{obs,2} vs c, not shown.](image1)

![Fig. 2 Desorption of avidin-FITC from biotin. The slopes of the two phase exponential kinetic resulted to the desorption rate constants k_{-3} and k_{-4} directly.](image2)
The deviation by 6 to 8 orders of magnitude from the expected $10^{15}$ M$^{-1}$ values for the affinity described in the literature [2] is probably due to steric hindrance of FITC-labelled avidin binding to the biotin surface.

References:

Development and Characterization of Novel Calcium and Magnesium Phosphate Biocements

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Introduction: The development of synthetic materials used for the filling of bone defects or replacement of surgically removed bone tissue requires accounting for several crucial material properties. The biocement has to be sterilizable, easy to handle, highly biocompatible, osteoconductive and osteostimulative. Furthermore it should sufficiently stabilize the bone defect and be resorbable over a conceivable period. The present study ties in with the recent state of knowledge concerning tricalcium phosphate (TCP) ceramics and particularly TCP cements and aims to further discover and enhance the bioresorptive properties of these. The main focus of the work was put on the mechanical activation of modified calcium and magnesium phosphate cements by powder milling.

Materials and Methods: Preparation of modified cements was carried out by sintering various mixtures of powders. Table 1 shows the composition of the examined cement products. The powders were suspended with isopropanol and stirred for one hour. After 3 days rest the dried mixtures were heated in a high temperature oven for 5 hours at 1100 °C. Verification of phase purity was carried out using X-Ray diffraction (XRD). Setting time of the cements was determined by means of a Gilmore needle. Compressive strength was tested using a static universal testing device.

Results and Discussion: The measurements showed that a small fraction of magnesium in a dicalcium phosphate (DCPD) and newberyite forming TCP cement leads to promising results regarding the suitability as bone replacement material and may serve as a good addition to the TCP cements already being used in clinical applications. The best results were obtained for an Mg_{2.25}Ca_{0.75}(PO_4)_2 based cement with citric acid as binder liquid. The combination of DCPD and newberyite leads to very good compressive strength, which was probably mainly achieved by the mechanical activation by 120 minutes of milling.

<table>
<thead>
<tr>
<th>Mg_{x}Ca_{y}(PO_4)_2</th>
<th>CaHPO_4 [mol]</th>
<th>CaCO_3 [mol]</th>
<th>MgHPO_4*3H_2O [mol]</th>
<th>Mg(OH)_2 [mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca_3(PO_4)_2</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ca_{2.25}Mg_{0.75}(PO_4)_2</td>
<td>1.5</td>
<td>0.75</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Ca_{1.5}Mg_{1.5}(PO_4)_2</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Ca_{0.75}Mg_{2.25}(PO_4)_2</td>
<td>0.5</td>
<td>0.25</td>
<td>1.5</td>
<td>0.75</td>
</tr>
<tr>
<td>Mg_3(PO_4)_2</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
Stents may be used to treat symptoms in patients suffering from malignant obstruction of the colon. The goal of this work is the study of the mechanical behaviour of a new design of colonic self-expandable metal (nitinol) stent. The stent geometry has a rhomboidal cell type, obtained expanding a nitinol slotted tube with longitudinal grooves in alternate disposition. The length and width of grooves determine structure flexibility in each zone. The stent reaches a high free expansion rate, with a longitudinal variable radial strength and a bell-shaped profile in the extremes in order to avoid migration. Using a FEM model is possible to simulate the mechanical requirements for this specific application. So is possible to calibrate parameters like the expansion force and radial compressive strength by mean of the size and disposition of the grooves in the tube. The finite element analysis allows knowing the response to peristaltic movement, which can be implemented in the numerical model by mean of the application of a pressure wave on the external surface of the stent structure. This analysis results in a better understanding of global mechanical behaviour of a new endoprosthesis for colonic strictures based on the superelasticity of the NiTi alloys.

References:

The proximal interphalangeal joint (PIP) is fundamental for the functional nature of the hand. The contracture in flexion of the PIP, secondary to traumatisms or illnesses leads to an important functional loss. The use of correcting splints is accepted as the chosen procedure for treating this problem. We have simulated the behaviour of a new splint based on the superelasticity of the NiTi alloy for the correction of joint deformities in fingers. It has a geometry which provides a controlled and adequate rigidity, in order of magnitude to other commercial orthosis, but with the advantage of a practically uniform restoring force in all of the range of its application.

A finite element analysis has been applied to the splint, considering a nonlinear model for the NiTi alloy, reproducing its resistance performance. In this way the design parameters can be adjusted by means of the simulation.

References:


The potential use of biosurfactants as contact lenses coating Agents

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During the last years, several applications of biosurfactants (BS) with medical purposes have been reported. BS are microbial compounds that exhibit pronounced surface and emulsifying activities, and have been considered relevant molecules for applications in combating many diseases and as therapeutic agents due to their antibacterial, antifungal and antiviral activities. Furthermore, their role as anti-adhesive agents against several pathogens illustrates their utility as suitable anti-adhesive coating agents for medical insertional materials (especially the ones produced of silicone rubber). Many of the adverse responses that occur during contact lenses (CL) wear are a consequence of its microbial colonization. Therefore, due to their properties, the use of BS as a coating agent could represent an alternative way of inhibiting or preventing contact lenses microbial adhesion. In previous work, it was already proven the antimicrobial and anti-adhesive activity of BS produced by several probiotic strains. However, although active against microbial adhesion, other contact lenses features, might limit the use of these BS. Thus, the purpose of this study was to evaluate the influence of BS adsorption on contact lenses refractive index (RI) and transmittance (T). Two silicone-hydrogel (Galyfilcon and Lotrafilcon B) and one conventional hydrogel CL (Etafilcon A) lenses were used. Three BS produced by different microbial sources were tested. Regarding the RI of silicone-hydrogel lenses with and without adsorbed biosurfactant, no differences were found. However, for the hydrogel CL conditioned with BS, higher RI was obtained as compared to the untreated CL. This increase in RI is a consequence of the dehydration observed with the adsorption of the BS, which is not desirable. All treated contact lenses types showed a decrease in transmittance levels in the visible spectra, being this effect more pronounced for higher BS concentrations as a result of the BS colour. Although the results obtained for the transmittance experiments are quite promising, further characterization/purification of the BS is required to enable the use of lower concentrations, more active and colorless fractions.
Design of smart Biocomposites for Bone Tissue Engineering Applications

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One basic research in Tissue Engineering is the development of “smart” multifunctional scaffolds for supporting and guiding the growth of cells. The ideal scaffold has to mimic ECM features and is supposed to be made of a biomaterial that provides all the necessary signals for the cells to grow, differentiate and interact forming the desired structure. In order to develop such a scaffold, matrices can be modified with various bioactive ligands enhancing cell adhesion, cell growth and differentiation.

In order to achieve a stable immobilization and a high biological activity of the ligands, covalent binding to spacer elements is preferred to direct adsorption to a matrix. Small linear biomolecules are applied as those spacer “arms” between the matrix and the ligands. In this way, a possible activity reduction of the bioactive molecules can be prevented which is known to occur at direct absorption.

In this study, polymers of vinylsaccharide N-methacrylamidoglucose (MAG) were used for this application. They were immobilized via adsorption on the matrix material Sponceram®, a macroporous ceramic doped with ZrO2. The desired ligands like growth factors, e.g. BMP-2 and cell adhesion promoting molecules were conjugated to the polymers by aldehyde chemistry. With regard to the application of the scaffolds in bone tissue engineering, the modified composite materials were tested in static cell culture with regard to cytotoxicity, using osteoblast precursor cells as a model cell line. In order to determine its differentiation inducing potential the composite was tested under dynamic conditions in a rotating bed bioreactor over a time period of four weeks. Histochemical analyses showed calcification of the ceramic. A performed PCR analysis showed the expression of bone specific markers. In summary, the developed composite materials showed promising attributes for the application in bone tissue engineering.
Change of properties of mononuclear leukocytes at patients with an osteogenesis imperfect after operative treatment with use nanosized hydroxylapatite coatings

T.V. Saprina, I.A. Khlusov, A.V. Karlov

Introduction: Patients with an osteogenesis imperfecta suffer from numerous fractures, an immobilization, improper functioning of an internals. Molecular mechanisms of this disease are insufficiently investigated. Clinical variants of disease are very polymorphic, showing from mild, subclinical forms, up to serious, and sometimes and lethal forms of disease. An active studying of nanosixed hydroxylapatite coverings (on the basis of calcium of phosphate) influence on a normal and pathological bone formation in vivo and in vitro is conducted in our center.

Aim: To define functional activity of mononuclear leukocytes and an opportunity of their differentiation to osteogenic cellular lines in patients with osteogenesis imperfecta at the presence of stimulators of a bone formation in vitro.

Material and methods: Mononuclear leukocytes were excreted by a standard method on a gradient of density (Phicoll-Paque, Sigma), discharged cells were incubated on the enriched medium for osteogenic cells Culture medium was studied for 2-d and 4-th day and concentrations of calcium, phosphorus, an alkaline phosphatase, osteocalcin, Cross-Laps were determined. Chosen cells were fixed and studied for 2-d and 4-th day accordingly.

Results: Principal differences in reactions of mononuclear leukocytes to presence of medium for cultivation of osteoblasts at patients with an osteogenesis imperfecta and healthy subjects are revealed. The Cross-Laps concentrations increased. from 14 up to 30 times at patients with an imperfect bone formation for 2 and 4 day of cultivation in culture medium. At healthy subjects concentration of this parameter did not variate. The following regularity was observed for all samples: the calcium concentration decreases and rising of phosphate were constant, maximum to 2-d day of cultivation. Collaterally with it, the maximum secretion of bone-specific alkaline phosphatase activity attained by 4-th day of cultivation of mononuclear leukocytes.

Morphological changes of cells showed in appearance of atypical "tapered" large cells, appearance of diverticulums of cytoplasm, cells with eccentrically posed nucleus. The following regularity was observed, that at patients with the most serious forms of disease, the amount of such cells was maximal. Such results testify the rising quantity of naïve mononuclear leucocytes in a periphery blood and about ability of them to be differentiated in osteogenic cells. These facts demand the further studying and identification received leucocytes (osteoblasts and/or osteoclasts).

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The covalent and non-covalent immobilization of growth factors such as recombinant human bone morphogenetic protein 2 (rhBMP-2) on metals and bone replacement materials in bioactive form is a recent development. Up to now the immobilization technology usually involved the chemical modification and activation of the biomaterial surface followed by attachment of the bioactive protein. Here we suggest an alternative method in which an affinity tag fused to an active protein will allow immobilization without additional chemistry.

For biomaterials such as minerals, metals (titanium, steel, CoCrMo), glass ceramics, teflon and possibly bone and teeth ideal adhesion molecules would be the foot proteins (Mefps) of the mussel M. edulis which contain the rare amino acid dihydroxy phenylalanine (DOPA). Recently it could be shown by Messersmith et al. that a single DOPA-molecule can be non-covalently bound to a titanium dioxide surface with a dissociation energy of 22.2 kcal/mol [3]. We therefore propose the DOPA-tag as a general and versatile affinity tag for the immobilization of proteins on biomaterials.

Up to now fusion tag proteins (poly His tag) are basically used for purification by highly specific metal ion chromatography [8]. One of the most important results of protein tag research is that with a few exceptions protein tags can react freely with surfaces or interfaces, such as chromatographic gels, biochips or biomaterials.

L-DOPA containing adhesive proteins in mussel foot proteins were first described by [2]. It has been shown by [3] that only a few DOPA residues are sufficient to anchor macromolecules non-covalently to titanium surfaces. Recently the preparation of protein tags based on DOPA-derivatives for the binding of bioactive proteins to biomaterials was suggested [9]. Here we show first results in the preparation of a poly-Tyr tagged rhBMP-2 which is to be converted into a poly-DOPA-tagged rhBMP-2.
Introduction: Computer based haptical models of biomedical tissues are commonly used to simulate and to practice surgical procedures, but they can also be used to simulate micro manipulations on cellular structures. These models, however, need an adequate biomechanical basis which allows a very precise simulation of the related tissue. Since these biomechanical models are very complex the simulations require much computer resources and a lot of time consuming computations, too. The human apperception, however, has the ability to psycho-optically compensate slight imprecise of haptical modelling. Therefore, it might be reasonable to simulate haptic tasks without extended bio-mechanical features of the involved cell. We developed a practicable haptic model of spherical cells.

Materials and Methods: We created a virtual model of a spherical cell and compared it to real cells following deformation and injection (cf. Fig.1A). A micropipette was vertically moved towards the cell membrane and deformed it for $z_0$ accordingly to the deformation force $F$. Hence, a deformation profile $z(r)$ occurred, while on the opposing site a co-deformation with radius $x_0$ could be observed (adapted to the surface of the cell fixation plate) and cell radius changes to $R$ (Fig. 1B). A raw haptic model created under radial-symmetric conditions is demonstrated in Fig. 1C. The haptic device used for the simulation procedure is shown in Fig. 1D.

We found a simple general mathematical equation to describe the deformation profile $z(r)$:

$$z(r) = \frac{z_0}{2} \left[1 + \cos \left(\pi \left(\frac{r}{x_0}\right)^p\right)\right], \quad 0 \leq |r| \leq x_0,$$

$z_0$ is the infiltration depth of the micropipette according to the deforming force $F$; the parameter $p$ reflects the elasto-mechanical characteristics of the membrane. All interactions performed with a pipette could be haptically simulated using the SensAble PHANTOM® Omni™ device by its 6 degrees of freedom. The visualization was processed with OpenGL using blending and texturing to increase the reality of the scene. The spherical cell was composed of 2500 nodes and its deformation was calculated with an (PC-) Intel Core 2 Duo 6600 processor in less than 1 ms. The deformation force $F$ was adapted to real experiments. Sun et al. [1] discovered that a force of 10 - 50 $\mu$N is needed to perforate the membrane e.g. of an oocyte. For a better handling and haptic experience of the interaction between the micropipette and the cell the simulated force was scaled by factor 1000.

Fig. 1: Mechanical deformation of a spherical cell. A: observed deformation (ICSI) used as a real example; B: deformation model (according to Sun’s model [1]); C: raw haptic model of the deformation based on a general deformation formula; D: Phantom® Omni™ haptic device used for the simulation.
Results: The presented models allowed us to perform a realistic simulation of spherical cell deformation, penetration and final withdrawing a micropipette using equation (1). As an example, Figure 2 shows some pictures of a simulated microinjection. After deformation of the cell membrane (Fig. 2.A) the pipette penetrated the membrane and was drawn to the centre of the cell (Fig. 2.B). Finally, the pipette was pulled out of the cell (Fig. 2.C). During this final procedure the cell showed a typical inversion profile which also could be modelled by formula (1) following a numerical adaption of parameter $p$.

Conclusion: It is shown, that the developed model allowed for a realistic haptic simulation scene of cell deformation and penetration. Both the actual geometrical form of the cell and its biomechanical behaviour could be expressed by only one parameter. The scaling of the force needed to deform and to perforate the membrane with the haptic system reflected diverse mechanical cell behaviour in realtime.

References

Influence of the educt concentrations on nano-hydroxyapatite crystal properties in the calcium hydroxide/orthophosphoric acid reaction system

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Introduction: Synthetic hydroxyapatite has been clinically used in a range of different forms including sintered ceramics, porous devices, granules and coatings. The efficient preparation of synthetic nano-crystalline hydroxyapatite (nano-HA) that mimics the structural features and properties of the mineral phase of natural hard tissue is a key target of biomaterial research. A common method for synthesizing nano-HA is based on the reaction of calcium hydroxide and orthophosphoric acid [1]. With regard to an up-scaling of this process, rather high educt concentrations are desired to increase the solid/liquid ratio. The influence of synthesis conditions, namely pH value, reaction temperature, Ca/P molar ratio, purity of educts, reactant addition rate, ripening time on morphology and crystallite dimensions of HA, has been intensively studied. Unfortunately, the influence of increasing educt concentrations on the crystallite dimensions and the chemical compositions of resulting nano-HA at larger batch amounts is difficult to interpret because in relevant studies [2, 3] different reaction temperatures were used and it is known that temperature influences crystal growth. It was the purpose of this work to study the influence of the educt concentrations on the properties of formed nano-HA. Phase-pure nano-HA amounts in a range between 19 and 400 g per batch were synthesized by stepwise increase of educt concentrations. Increasing the educt concentrations, the Ca/P molar ratios decreased from 1.79 to 1.68. No calcium-deficient HA were detected. Quantitative phase compositions determined by Rietveld refinements showed that the nano-HA powders consist of highly crystalline HA with amorphous contents below 10 wt%. Neither additional crystalline phases nor differences in reflex locations or in reflex widths among synthesized, unsintered nano-HA were detected in the XRD patterns. After sintering small traces of lime were found in the patterns which is typical for HA with a Ca/P ratio larger than 1.67. Specific surface area measurements, Rietveld refinements and TEM investigations showed that the crystallite properties (structure, crystal size and shape) of the formed nano-HA were not markedly changed by increasing the educt concentrations. TEM images showed well crystallized nano-crystallites, elongated in crystallographic c direction with average crystallite dimensions of 20 to 40 nm in width and 60 to 100 nm in length. It may concluded that under the selected reaction conditions,
the supersaturation created in the reaction medium, is high enough to produce a large number of direct nucleation centres simultaneously accomplished with a suppression of crystal growth of precipitated nano-HA crystallites. In summary, the established Ca(OH)$_2$/H$_3$PO$_4$ synthesis procedure represents a simple, cost-effective method to synthesize larger amounts of phase-pure, highly crystalline nano-HA with reproducible crystallite sizes and morphology usable in biomedical applications.

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References:

MICROSTRUCTURE AND PROPERTIES OF NANOSTRUCTURED TITANIUM FOR MEDICAL APLICATION

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Results of the microstructure and mechanical properties investigations of ultra-fine grained and nanostructured pure titanium are presented. The titanium samples in ultra-fine grained state were produced by multiple uniaxial pressing (\textit{abc}-pressing), subsequent rolling and recrystallization annealing. It was shown that the method of the titanium samples preparation leads to the formation of the uniform and ultra-fine grained structure that is thermo stabled up to 350 °C with the average size of the structural elements (grain, subgrain, fragment) less than 100 nm. It was established that formed titanium has not only high microhardness (3400 MPa), but also highly static strength (yield strength is 1100 MPa and strength is 1160 MPa) and the satisfactory plasticity (up to 6% at uniaxial tension). It was established that produced titanium samples show high mechanical properties as a result of the ultra-fine grained structure formation in titanium. The mechanical properties of the formed ultra-fine grained titanium samples are comparable with high-strength alloys (Ti6Al4V). It was shown that the formed high-strength ultra-fine grained titanium is successfully used to produce the dental intraosteo screw implants with original design.
**Biocompatible calcium phosphate coatings with different Ca/P ratio and structure deposited by rf-magnetron sputtering**

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**Introduction:** The main problems associated with medical implants can be overcome or reduced by the deposition of biocompatible calcium phosphate coatings. Many methods were developed that can be used to obtain such a coating with the necessary elemental and phase composition [1, 2], but some problems still exist. Therefore, this work is devoted to the thorough characterisation of the coatings deposited by rf-magnetron sputtering.

**Materials and Methods:** As substrates we used technically pure titanium, the nickel titanium alloy NiTi, and stainless steel 316L. To describe a plasma composition we used Optical Emission Spectroscopy (OES). To investigate the properties of a target material and coatings deposited, we used scanning electron microscopy (SEM), X-ray diffractometry, IR-spectroscopy, and the scratch test method. Moreover, atomic absorption spectroscopy was used to study the nickel release rate from NiTi substrates into water and saline solution 0.9% NaCl; biological tests (*in vivo* and *in vitro*) were also performed. As a target material we used stoichiometric hydroxyapatite (molar Ca/P ratio 1.67). Calcium phosphate coatings were deposited under different experimental conditions such as working gas atmosphere (argon, oxygen, gaseous mixture of argon and oxygen), negative substrate biases, rf-power, and deposition time. The maximal thickness of the coatings did not exceed 3 µm.

**Results and discussion:** As-deposited calcium phosphate coatings morphology reflected the morphology of the underlying substrate. The coatings were dense and pore-free without any visible defects and cracks. By IR-spectroscopy, the bands belonging to hydroxyapatite were found (P-O and O-H). The deposition process resulted in absence of the part of the hydroxyl groups in crystalline lattice of the coatings according to IR-spectra; in this case, presumably oxide anions occupy these sites to preserve lattice electroneutrality. The composition of the coatings did not depend on the deposition procedure and the substrate used. However, the variation of the deposition parameters led to a change in Ca/P ratio, coating crystallinity and coating thickness. The Ca/P ratio could be changed from 1.53 to 3.88 by varying the deposition procedure. Moreover, amorphous or crystalline hydroxyapatite coatings were deposited with Ca/P ratio between aforementioned values. The calcium phosphate coating structure depended on the magnitude of applied rf-power, i.e. low and high power levels of <0.1 W cm⁻² and >1 W cm⁻² resulted in amorphous or crystalline coating structures, respectively. We also found that crystalline hydroxyapatite coatings reduced the nickel release rate from NiTi by a factor of 7 to 11 compared to the bare substrate. Amorphous coatings did not reduce the release rate of nickel because of their high dissolution rate in the immersion medium (Table 1).

**Table 1.** The nickel release rate calculated for coated and uncoated NiTi substrates.

<table>
<thead>
<tr>
<th>Deposition procedure</th>
<th>Average nickel ion release rate (ng cm⁻² day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>distilled water</td>
</tr>
<tr>
<td>290 W (argon or oxygen)</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>30 W (argon or oxygen)</td>
<td>3.6±0.3</td>
</tr>
<tr>
<td>NiTi without Ca/P coating</td>
<td>4.4±0.3</td>
</tr>
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</table>

Scratch test investigations revealed that calcium-phosphate coatings with a thickness of up to 1.6 µm combines an optimal value of adhesion strength and cohesive resistance that did not depend on the substrate used. Biological investigations showed that implants with calcium phosphate coatings were nontoxic, i.e. no signs of inflammatory or infection reactions were observed after experiments on mice (Balb/c) during 45 days. Thus, rf-magnetron sputtering is a promising technique to obtain a dense and pore-free calcium phosphate coating with different Ca/P ratio and structure (amorphous and crystalline).

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Silicon-substituted hydroxyapatite coatings deposited by rf-magnetron sputtering

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Introduction: Nowadays, many research works are carried out to improve the bioactive properties of hydroxyapatite. One way is chemical modification of apatite. Ions of silicon oxide $\text{SiO}_4^{4-}$ is suggested to be a possible modifier of synthetic hydroxyapatite [1], whereas Rf-magnetron sputtering is one of the techniques used to deposit thin silicon-substituted hydroxyapatite (Si-HA) coating because it allows to prepare coating with high adhesion strength to different metallic substrates [2]. The aim of this work is to deposit silicon-substituted hydroxyapatite (Si-HA) coatings by rf-magnetron sputtering and investigate their properties.

Materials and Methods: An installation for surface modification 08PKHO-100T-005 (5.28MHz) with a magnetron device was used to deposit Si-HA coatings. As substrates we used stainless steel 316L, titanium. The powder of silicon-substituted hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_{6-x}\text{SiO}_4\text{OH}2-x$, $x=0,25$) was synthesized by the method of mechanochemistry and it was used as a precursor to prepare a target for sputtering. To investigate the properties of coatings deposited there were used such techniques as Scanning Electron Microscopy (SEM), X-ray diffractometry (XRD), Fourier IR-spectroscopy, Scratch test method.

Results and discussion: The surface of Si-HA coatings is dense, pore-free without any visible defects and cracks. According to the data obtained by Energy Dispersive x-ray analysis (EDAX) Si-HA coating consists of C – carbon, Ca – calcium, P – phosphorus, O – oxygen and Si – silicon. Moreover, the results indicated that the content of Si is about 1 %. The structure of the coatings is single-phase with (002) preferred orientation that was assigned to hydroxyapatite. The FTIR-spectra of the coatings showed the presence of all absorption bands corresponded to hydroxyapatite at (i) 1000–1100 and 564 cm$^{-1}$ (asymmetric stretching and asymmetric bending vibrations of $\text{PO}_4^{3-}$ groups), (ii) 1400–1500 cm$^{-1}$ (asymmetric stretching vibration of $\text{CO}_3^{2-}$ groups), and (iii) 3575 cm$^{-1}$ (stretching vibration of the $\text{OH}^-$ groups). Additionally, a band appearing at 870 cm$^{-1}$ is assigned to $\text{Si}-\text{O}$ vibration modes of $\text{SiO}_4^{4-}$ groups. The investigation of adhesion strength between the Si-HA coating and the substrate did not show signs of disruption in the vicinity of the scratch, and the coating did not exfoliate even at a maximal load of 2 N. Though the indenter penetrated into the substrate, no traces of bursting of the coating along a scratching direction were found.

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References:
FLOCCULATION BEHAVIOUR OF POLYMER BRUSHES

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Introduction: Dextran are essential natural polymers in biomedical, agricultural, photographic and other areas. Modified Polysaccharides are perspective materials and have many applications in various fields. Modification by grafting is a promising approach to obtain new materials with special properties and possibilities for industrial and biomedical applications. Water-soluble synthetic polymers grafted to Polysaccharide backbone can be used to influence the colloidal stability of particles in solution therefore are perspective materials for flocculation performance \cite{1}, nanoscale metal catalysts stabilizing \cite{2}, in the controlled encapsulation and so forth. It was shown that graft copolymers with Polysaccharide backbone are highly effective biodegradable flocculants \cite{1-3}, their flocculation activity depends on their macromolecular structure \cite{3}. So, designing of such materials is quite important problem to solve specific tasks.

Fig. 1. Photo of the sedimentation of blank kaolin suspension (on the left) and suspension with D-PAA copolymer added (on the right) in 1 min since the test beginning.

Materials and Methods: The copolymers Dextran-graft-Polyacrylamide (D-g-PAA) and Dextran Sulphate-graft-Polyacrylamide (SD-g-PAA) with long polysaccharide backbone ($M_w = 500\,000$) and different number of grafted PAA-chains (25 and 50) of various length were synthesized by radical copolymerization. Ceric-ion-induced redox initiation method has been used for the synthesis. The conditions of synthesis were described in \cite{4}. Linear polyacrylamides PAA1 and PAA2 with average molar weight $M_w = 1 \times 10^6$ and $M_w = 2 \times 10^6$ relatively was also synthesized using the cerium initiation method.

The copolymers and polyacrylamides were identified and characterized by elemental analysis, $^1$H NMR, self-exclusion chromatography, light scattering and viscometry methods.

Results and Discussion: The flocculation tests clearly indicated that all the polymers studied have good flocculation abilities to be added in very small concentrations. In the presence of D-PAA and SD-PAA copolymers the kaolin particles are aggregated in visible flocks and the sedimentation rate is significantly increased (Fig. 1). The plots of suspension interface motion show that the flock sedimentation rates depend on the flocculant concentration applied. The sedimentation plots also suggest that the copolymers D-PAA and SD-PAA with longer grafts are more effective flocculants.

The most important parameter of the flocculation efficiency is the supernatant clarity. All the graft copolymers are more effective flocculants than the linear PAA. The apparent cause is that the graft copolymers have a non-linear structure resulting in more expanded conformations of the macromolecules in solution. The comparison of the supernatant clarity data indicates that the copolymers having longer PAA-grafts are more effective flocculants. Obviously, increase in length of grafted chains causes the better availability of functional groups of PAA-chains to link fine kaolin particles owing to most extended conformation of grafted PAA-chains in this case.

Acknowledgements
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Reagent-free covalent crosslinking of chitosan-gelatin films for medical applications

A. Zakhariouta and A. Taralp

Introduction: Chitosan-gelatin composites show promise in tissue engineering and drug delivery for reasons related to their intrinsic biocompatibility, biodegradability, biocidal activity, and easy preparation in forms spanning diverse mechanical properties, porosities and interconnectivities [1, 2]. Regarded as a soft polymer, the incorporation of Chitosan & gelatin in 1-2% acetic acid (sucrose added in some samples) Flash-freezing & subsequent lyophilization (12h) ↓ Lyophilized chitosan-gelatin films Optimization of ionization states (8h) & subsequent in vacuo heating (100°C, 14h) ↓ Crosslinked composite precursor film Work-up in PBS, washing with dH2O, re-freezing & re-drying ↓ Porous inter-connected base film PBS or trypsin ↓ DMA ↓ Stability & mechanical analysis results of such formulations in mechanically challenging applications necessitates the use of established crosslinking agents such as formaldehyde and glutaraldehyde, amongst others [3, 4]. While effective, reagent-based strategies carry along concerns related to the direct toxicity of many crosslinkers and to the potential or established indirect toxicity posed by various metabolic byproducts arising during in vivo degradation. It follows to reason that a reagent-free crosslinking approach, namely, the low-pressure thermal dehydration of ammonium carboxylate salt bridges, could provide a means to form intermolecular amide bonds while avoiding the risks mentioned above. In view of the high structural stability of proteins in powder form, this method had already shown utility to desolubilize protein lyophilisates, presumably by cross-linking some or many of the bridging ammonium carboxylate groups [5]. This plausible bonding mode was confirmed directly in later work using lyophilized proteins [6]. In all cases, bonds were formed at relatively low temperatures (i.e., 75-120°C) by means of a vacuum to better drive off water. In this study (vide chart, left), an identical rationale and similar in vacuo thermal treatment was used to crosslink composite chitosan-gelatin films. As such, this concept could facilitate the creation of safer and potentially more easily legislated reagent-free biomedical products such as wound dressings.

Rationale: This technique can be extended to crosslink chitosan-gelatin composites according to: Gelatin-COO⁻ + NH₃-Chitosan → Gelatin-CONH-Chitosan + H₂O(g) and Gelatin-COO⁻ +NH₃-Gelatin → Gelatin-CONH-Gelatin + H₂O(g). In reviewing the structure of chitosan, in vacuo thermally-induced amidation (and possibly esterification) describe the only mode(s) of covalent crosslinking to gelatin. The possibility of achieving crosslinking by these modes was supported by SDS-PAGE analyses of in vacuo thermally-treated albumin lyophilisates (not shown), which indicated crosslinking in view that thermally pre-treated albumins could not enter the gel matrix. Since mercaptans and SDS were present when the proteins were boiled in PAGE sample buffer, other potential modes of intermolecular interaction, such as disulfide or hydrophobic bonding, were ruled out. Still other studies on proteins had established the direct contribution of inter-protein amide bonding and had implied the possibility of inter-protein ester bonding [5,6].

Materials & Methods: Stock solutions: Chitosan (2-3wt%) was dissolved in dilute acetic acid (1-2wt%, 50-60°C) under vigorous agitation. Once homogeneity was established, gelatin (2-6wt%) was added and dissolution was continued under mild agitation. Finally, sucrose (0-10wt%) was added and dissolved under mild agitation.

Film preparation: Warm aliquots (5ml) of stock solution were poured into polystyrene Petri dishes and allowed to cool. The Petri dish and contents were subsequently submerged in liquid nitrogen. Frozen samples were lyophilized (0.02-0.04mbar, 12h) without applying external cooling. Prior to in vacuo heating, the lyophilized films were incubated
(RT, 8–12h) in a partially-evacuated desiccator containing solid ammonium carbonate (5g).

**In vacuo thermal crosslinking:** A makeshift apparatus consisting of an evacuatable glass vessel and variable heat source was employed to drive the in vacuo crosslinking. Lyophilized samples were transferred into the glass vessel and incubated (100°C, 0.2-0.6mbar, 14h). To remove porogens and/or unreacted chitosan and/or gelatin, the crosslinked films were soaked (12h) in phosphate buffered saline (PBS) buffer, pH 7.4, and washed using distilled water. Finally, the samples flash frozen again and re-dried under vacuum in a bell jar.

**Scanning electron microscopic structural analysis:** Samples were frozen in liquid nitrogen and cut with a fresh razor blade before treatment with a carbon coater. A gun voltage of 2kV was employed.

**Stability tests:** Solubility - Crosslinked films and non-heated controls were agitated in PBS buffer (1wt%, 37°C, 450rpm, variable time). SEM images of washed and re-dried samples were obtained. Biodegradation - Samples (5mg) were cut and incubated (37°C, 450rpm, variable time) in PBS buffer (1ml) containing trypsin (310 USP/ml). Ninhydrin analysis was performed using spent buffer/ninhydrin/isopropanol comprising 100:1:99[v/v/v] (70°C, 20min). Color yields were analyzed spectrophotometrically (570nm). All comparisons were made against the non-crosslinked control, which was arbitrarily assigned a color yield of 100%. SEM images of washed and re-dried samples were obtained.

**Porosity:** Porosity was estimated from the weight and dimensions of at least 6 samples acquired from different regions of each film. Samples were incubated in ethanol (30min, partial vacuum) until permeated. Excess ethanol was wiped from the surface with filter paper and the samples were re-weighed. Porosity (%) was found according to 100% x ((W_w-W_d)/\rho_{EtOH})/V where W_d & W_w are dry and ethanol-perfused weights, V is film volume and \rho_{EtOH} is ethanol density [1].

**Mechanical testing:** Rectangular samples (1mm×5mm×10mm) were cut and strained against dynamic tension (35-37°C; 1Hz). Analysis was performed on dry and ethanol-perfused samples (i.e., suspended in ethanol under partial vacuum, 30min). NETZSCH DMA 242 software was employed to evaluate the results.

**Results & Discussion:** The potential merit of this in vacuo thermal dehydration strategy was exemplified in the course of preparing crosslinked chitosan-gelatin films with a porous, scaffold-like structure. With advantages foreseen in wound dressing applications, such as inherent antimicrobial activity and zero reagent toxicity, it appears that such a method can provide an alternative and reagent-free means to crosslink biomaterials and to effect changes of solubility, biodegradability and mechanical properties.

**Fig. 1.** Morphology of crosslinked chitosan-gelatin film (2wt%: 4wt%: 2wt% sucrose) before (left) and after (right) incubation (1wk) in PBS (37°C). Horizontal frame = 470 m.

**Fig. 2.** Morphology of crosslinked chitosan-gelatin film (3wt%: 6wt%) before (left) and after (right) incubation (37°C, 14h) in trypsin solution. Horizontal frame = 330 m.

**Fig. 3.** Ninhydrin-monitored liberation of primary amino groups from crosslinked chitosan-gelatin (3wt%: 6wt%) during tryptic digestion (37°C, 14h).

SEM analyses showed that thermally treated films bore considerable porosity and interconnectivity in the presence or absence of sucrose as porogen (Figs. 1 vs. 2). While
lyophilized non-heated control films quickly dispersed into PBS buffer (not shown), thermally crosslinked films remained intact following a month of incubation under the same conditions (Fig. 1, right). That being said, the crosslinked films did exhibit some swelling and concomitant flexibility upon rehydration in PBS. Tryptic activity caused minor erosion of the crosslinked films. In particular, the “surface polishing” effect of trypsin was clear in pitting the appearance of a trypsin-treated film against its zero time control (Fig. 2). A time-course tryptic digest profile (Fig. 3) also indicated that crosslinked films had resisted but not prevented hydrolysis. Non-crosslinked controls were quickly degraded by trypsin (not shown). Porosity measurements and DMA-type mechanical tests were performed using ethanol-perfused films in preference to hydrated films. Like water, ethanol imparted some necessary mechanical compliancy to the film. However, ethanol was also found to limit porosity calculation errors attributed to film volume changes upon resolvation. The porosity of thermally-treated films ranged from 82-89% depending on the chitosan, gelatin and sucrose concentrations. DMA testing of non-crosslinked dry controls proved unsuccessful as these samples were too fragile to analyze (not shown). Crosslinked dry samples proved highly inflexible (0.5% elongation; max. dynamic load = 3N), with an elastic modulus largely exceeding the set maximum of 30MPa. Not surprisingly, ethanol-perfused crosslinked films and ethanol-perfused non-crosslinked controls strained to a much greater extent under dynamic tension (8-15% elongation) but lacked the toughness of the crosslinked dry samples. For instance, one chitosan-gelatin sample (3wt%: 6wt%) that was strained to failure (max. dynamic load = 1N) displayed 10% elongation and an elastic modulus of 30MPa. Such traits appeared to satisfy some performance prerequisites of wound dressings. Work is currently underway to assess the potential of this material in wound healing and cell proliferation.

**Summary:** In light of the current results, it appears that these films may not only facilitate skin-localized therapies but they could also satisfy the function, morphology, mechanical properties and biodegradability expectations of the more challenging tissue scaffolds. It also follows to reason that the scope of this reagent-free crosslinking method can be extended to incorporate different protein-based components in order to facilitate biochemical processes such as O₂ transfer or directed cell growth, and to help prepare other specialty products such as polyhemoglobin and antibody conjugates.

**References:**


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INTELLIGENT MODULAR NETWORK ORTHOPEDIC DEVICE

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The direction of our study was focused on the sub-systems that ensure the protection and recovery of traumatized bones by absorbing the external forces and moments that the normal bone is subject to. This category of implants falls into the category of external attaching sub-systems and they have the following advantages:

- minimal intervention and small size of the implanted foreign bodies – only the rods are introduced into the bone structure
- the small incisions mean smaller risk of infection
- the mechanical load of the system can be distributed according to a tensiometric map which optimally favors the healing process
- the possibility of dynamically modifying the load map by external intervention, without any other traumatizing interventions on the patient
- blood edema and improves bone recovery and vascularization of the region.

The actual external fixators have to be manually adjusted with respect to the main axis of the bone. Unfortunately, the degrees of freedom of current devices are limited to 3 or 4 vertical screws. The solution to these problems is the Modular Adaptive Implant - MAI.

A modular net, identical in structure with the bone and locally configurable in terms of tension and release, is best design solution in terms of biocompatibility. The identification of the mechanical solicitation of the particular bone structure, using finite element method, leads to the concept of the practical implementation of a feasible device able to undertake the functionality of normal bones. This device will partially discharge the tensions in the fractured bones (the fractured parts still need to be tensioned to allow the formation of the callus) improving the recovery time and the healing conditions.

The proposed intelligent device has a network structure, with modules made out of Nitinol, especially designed in order to ensure a rapid connection and/or extraction of one or more MAI modules. The binding of the SMA modules ensures the same function as other immobilization devices, but also respects additional conditions concerning variable tension and its discharge. Moreover, these modules allow little movement in the alignment of the fractured parts, reducing the risks of wrong orientation or additional bones callus.

In order to identify the optimal design, different implants were developed and experimented using numerical simulation. Our research focused on identifying optimal shapes which would permit not only previous mechanical tensioning, but also a rapid change of modules tensed at a certain value with other modules with an elastic module that is superior or inferior to the previous one, depending on the desired protective or recovery-oriented tension map. We also designed and experimented numerically and physically a series of modules both individually and in a network. The following figures show the main types of experimental modules, in a relative order of betterment of the concept and design.

Fig 1. Healthy bone

The proper shape of MAI is related to the bones microscopic structure and to the numerical simulation presented in the previous chapter. As one can observe, the internal architecture of the healthy bone has a regular modular structure.
We suggest that the Variant 6 for the unitary SMA module structure is an optimum design which ensures not only the stability of the super-elastic network and constant force requirements, but also a rapid coupling/decoupling procedure. This solution respects the protection of the patients for accidental unusual mechanical tension. Using Solid Works package and COSMOS software we proceed to various numerical simulation of SMA module, in order to test the proper mechanical design.

Doctors can use SMA modules with different internal reaction tension, but all the modules will have same shape and dimension. The connection with affected bones and the support for this net are similar to those of a classic external fixator, but allowing for the advantages of minimal invasive techniques. The new device leads to a simple post-operative training program of the patient. The relative advanced movement independence of patient with MAI network apparatus can lead to possibility of short distance walking.

The following pictures show experimental network versions obtained by modules created using a rapid prototyping technology—a 3D Zcorp Printer, a infrastructure obtained from the financial support of this project.
AFM and SEM investigation of gold nanoparticles produced by periodical point contact loading


We have shown that separate gold nanoparticles can be produced by periodical loading of metallic substrates with a gold pin at point contact in air as well as in liquids. The resulting surface is quite heterogeneous requiring microscopy on microscale as well as on nanoscale.

We have investigated morphological fine structure and size distribution of the particles produced. Scanning electron as well as atomic force microscopy showed that the observed objects consist of 20 nm particles which are assumed to be result of mechanical breaking of cold spot welded nanocontacts. We have found that the lateral density of deposited on substrate particles produced in liquids decreases with distance from the center of the contact. This indicates the nanoparticles stem from the point contact area.

A possibility of nanoparticles production in protein solutions should be checked.
Development of photosensitive and photorecording systems of high resolving power is one of the most important tasks for eye implant technique. At present, resolving power of photorecording systems is restricted by dimension of separate photosensitive element of corresponding matrix. This dimension already is close to its technical limit, while each such element should be connected with separate conductor of matrix. In other words, resolving power of matrix photorecording elements is not enough for eye implant technique, and development of new approaches to increasing of this parameter is important task.

We propose a new scheme of recording of optical signals of high resolving power in present report. This scheme gives possibility to achieve resolving power comparable with the theoretical limit, which is giving by wavelength of optical irradiation. Proposed approach is based on multi-channel scheme of light recording (Fig.1). Light sources 1-5 produces fixed distributions of light intensities of fixed profile. Each of such profiles corresponds to one of Radamacher’s functions. It is known well, that set of productions of such functions forms full system of Walsh’s function basis in accordance with formula:

\[ Wal_j(x) = (Rad_1(x))^j (Rad_2(x))^j \ldots (Rad_n(x))^j \]

where indexes \( j_m \) is equal independently 0 or 1.

Photosensitive layer 6 carries out calculation of production (1). Similar layer 7 allowing calculation of integral

\[ R_j \propto \int_{-a/2}^{a/2} Wal_j(x) J_1(x) dx \]

which, obviously represent a Fourier transformation of input optical distribution \( J_1(x) \). Full information about decomposition of input distribution in Fourier row will be obtained by changing indexes \( j_m \) i.e. by switching on and off sources 1-5. Thus, resolving power of proposed method is restricted by accuracy of generation of distributions corresponding to functions 1-5 only, while there are no necessity to decompose an image on separate poits for photorecording. Holography methods allow achievement of accuracy comparable with wavelength. Additionally, using of new polymer intelligent materials [1] gives possibility to direct calculation of production (1) with rather high number of functions 1-5.

Reference:
