Bioresorption of Bone Substitutes Based on Calcium Phosphates

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As regenerative bone therapies are developing in the fields of maxillo-facial, orthopedic and spine surgeries, bioresorbable bone substitutes attract a growing interest. Resorbable polymers such as poly(L-lactide) (PLLA), poly(glycolide) (PGA) and mixtures thereof have long been used for the manufacture of osteosynthesis plates and screws ; more recently, these materials have been used either for making macroporous bone substitutes, or for preparing 3D macroporous templates for bone tissue engineering.

The use of bone substitutes based on calcium phosphates, in the form either of macroporous ceramic blocks or of injectable cement pastes of various compositions, has also gained polularity during the recent years. New developments are now focusing on polymer-matrix composites reinforced with nanocrystalline hydroxyapatite, thus attempting to mimick the composition and mechanical properties of natural bone.

The bioresorption process of bone substitutes obviously depends on their chemical nature. In contrast with PLLA and PGA, the resorption of which relies on their spontaneous hydrolysis and subsequent depolymerization in aqueous media, the resorption of calcium-phosphate substitutes is closely related with their solubility in aqueous solutions.

Thermodynamic simulations show that among the calcium phosphates used in the preparation of ceramic and cementitious bone substitutes, only mono-calcium phosphate monohydrate (MCPM), di-calcium phosphate dihydrate (DCPD, *alias* brushite) and octa-calcium phosphate (OCP) can dissolve spontaneously in normal physiological conditions (37°C, pH = 7.4). Other typical compouds, such as hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP), can only dissolve in more acidic conditions that occur during acute inflammatory reactions, or result from osteclastic activity.

The bioresorption of calcium phosphate bone substitutes (cements and macroporous ceramics) will be discussed in the light of their physico-chemical interactions with the physiological environment during bone remodeling

Biocompatible, biofunctional bioresorbable polymers for temporary therapeutic applications: The Concept of Artificial Biopolymers

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Historically, the search for polymeric systems that can better respect living systems in applications requiring a biomaterial for a limited period of time, started in the late sixties with the development of the so-called "bioabsorbable sutures" based on glycolic and lactic acids. As early as 1974, J. Leray and myself became interested in the use of synthetic polymers derived from hydroxyl-2 ethyl methacrylate after the pioneering work of G. Winter who showed that sponges of the Hydron®-type promoted calcification *in vivo* (1). One of the shortcomings of this type of sponges was their poor mechanical properties and resistance to compression.

For the sake of circumventing this shortcoming, we came to the idea that degradable polymers could behave like a sponge, degradation forming pores schematically.

Starting from earlier work dealing with sutures and mandibula osteosynthesis, we selected lactic and glycolic acid-derived aliphatic polyesters (PLAxGAy where x and y stand for the percentage of L-lactyl and glycolyl units, respectively) as candidates. However, after a short period of preliminary animal investigations with L. Sedel and P. Christel, it appeared necessary to revisit the polymerisation processes in order to improve very much both intrinsic mechanical strength and lifetime to match the requirements of osteosynthesis.

This period led us to select PLA98 for practical reasons that will be emphasised (2). The first application in human was performed in 1981, thanks to a traumatologist, Dr M. Audion. A company, Phusis, was created in 1984, thanks to A. Tornier, to develop bioresorbable materials for effective bone surgery. The commercial success came only on 1990 under the form of an interference screw (3).

It is later on that we justified this choice after the identification of the number of factors that affect the hydrolytic degradation of PLAxGAy polymers, such as chemical, physical and configurational structures, polymerisation initiator and also size (4). From there, other devices were developed (5).

It is from the success of lactic and glycolic-derived polymers that we introduced the concept of artificial biopolymers, i.e. of non-natural bioresorbable polymers made of building blocks that are normally present in biochemical pathways and that generate metabolites upon degradation or biodegradation. Accordingly, degradation by-products are biocompatible and can be eliminated and/or bioassimilated via natural pathways.

As a consequence, we introduced polymers like poly(β-malic acid)s, poly(L-lysine citramide), poly(amino serinate and various copolymers (6), thus extending the fields of applications to functional bioresorbable polymers and polymer therapeutics, including drug delivery and drug targeting, domains that are still at the pioneering stage.

However, because of the high therapeutic potential of PLAxGAy polymers, a demand appeared recently for methods aimed at modifying their intrinsic properties. The anionic activation of aliphatic polyesters using lithium di-isopropyl amidure (LDA) was introduced to make PLAxGAy surfaces functional and to create novel artificial biopolymers from poly(ε-caprolactone). Functionalisation of lactic and glycolic-derived polymers was also achieved by introducing gluconyl residues by copolymerisation. This route provides a original means to perform chemistry on PLAxGAy-type macromolecules (6).

Recently, we approached the field of tissue engineering starting from the point that matching the degradation rate of the scaffold with the rate of tissue growth or tissue reconstruction is a key factor. The strategy was applied to skin culture and reconstruction. Our first approach was based again on PLAxGAy polymers. However, it was found that glycolic acid is unfavourable to keratinocyte growth whereas it does not affect the growth of other cell types. Once again PLAx appeared performing better in this type of application (7).

According to the same strategy, we recently challenged the problem of bioresorbable stenting of coronary arteries. It was shown that PLAx-based stent prototype can be implanted in rabbit according to angioplasty and stenting in human (8).

At the present time, the future of artificial biopolymers appears promising in surgery, in pharmacology and in tissue reconstruction. However, the most open domain is probably that of the medication of bioresorbable surgical devices made of artificial biopolymers and antibiotics, antitumoral agents, growth factors, etc., as already done with non-degradable polymers. A few more years are necessary to reach that ambitious stage.

Acknowledgements

The authors is grateful to his numerous co-workers and partners who contributed to the work exposed herein.

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Development of Superparamagnetic Nanoparticles for Magnetic Imaging and as Drug Vectors for Therapy

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The use of Super Paramagnetic Iron Oxide Nanoparticles (**SPIONs**) (Fig.1) combined with MRI is under clinical evaluation to enhance detection of neurodegenerative diseases.

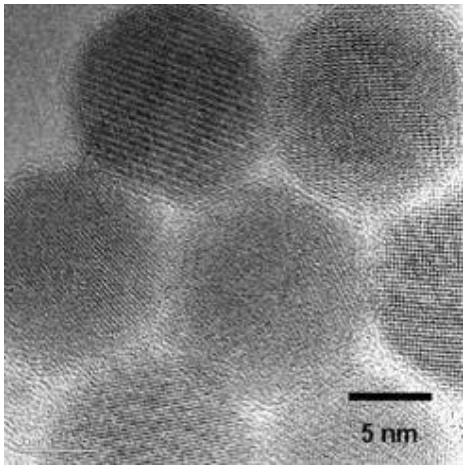

Fig. 1: Transmission electron micrograph (TEM) showing iron oxide particles in the 10 nm range.

A major improvement would be to link therapeutic drugs to the SPIONs to achieve targeted drug delivery, either at the cell surface or intracellularly, together with active disease detection, without inducing cell reaction. Our objectives are to define the characteristics of SPIONS able to achieve cell-specific interaction with brain-derived structures.

Our system consists in an ironoxide core (9-10 nm diameter) coated either with polyvinylalcohol (PVA) (native nanoparticles) or with PVA which has been functionalized on the hydroxyl groups with either amino (aminoPVA), carboxylate (carboxylatePVA) or thiol (thiolPVA) groups. We investigated the cellular uptake, the cytotoxicity and the interaction of these various nanoparticles with brain-derived endothelial cells, microglial cells and differentiating 3-dimensional aggregates.

Only aminoPVA-SPIONs were taken up by brain cells, but did not invade brain cell aggregates lower than the first cell layer. Fluorescent aminoPVA-SPIONs demonstrated cell interaction with brain-derived endothelial and microglial cells and intracellular uptake by microglial cells using confocal microscopy. No cytotoxicity or inflammatory reaction was observed as determined by cell respiratory potential, death, the production of the inflammatory mediator nitric oxide or the expression of inflammatory markers. AminoPVA-SPIONs neither invaded brain cell aggregates lower than the first cell layer nor induced microglial cell activation in the aggregates.

In order to develop therapeutics-derivatized-SPIONs and to couple defined molecules for targeted detection and drug-delivery purposes, we have designed and synthesized a multivalent linker, to which drugs are covalently coupled via a biologically labile linkage before the preparation of derivatized SPIONs (Fig 2).

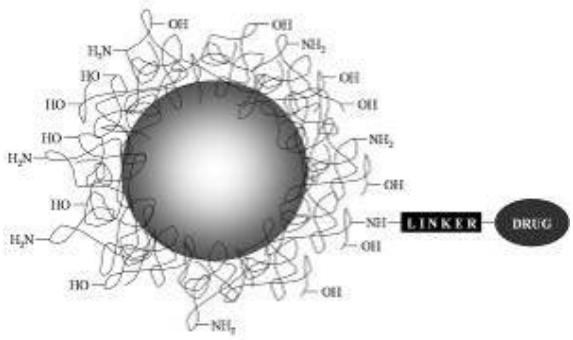

Fig. 2: Model SPIONs under evaluation, with a linker coupled to a therapeutic drug

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Medium-chain-length polyhydroxyalkanoate: a bacterial biopolyester for medical applications?

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INTRODUCTION: Medium-chain-length poly([*R*]-3-hydroxyalkanoate) (mclPHA, monomers from C_6-C_{14}) is a water insoluble, biodegradable, and biocompatible class of biopolyesters of microbial origin. Its use for medical applications has been proposed[1] but due to the limited availability only a few studies have been carried out[2].

A delicate point is the possible contamination of mclPHA with endotoxins due to its production in Gram negative bacteria. To date, chlorinated solvents have been used to extract mclPHA from freeze-dried biomass. A novel method has been developed that allowed extraction and reduction of contaminations using green chemistry.

In this work the interaction between mclPHAs and 3T3 fibroblasts or human bone marrow cells (HBMCs) was studied. The attention was turned to cell adhesion and polymer degradation.

METHODS: Three different mclPHAs were produced in *Pseudomonas putida* GPo1 and extracted using non-halogenated solvents. The monomer composition of the Poly(3-hydroxy-octanoate-*co*-3-hydroxy-10-undecenoates) was 100/0 w% (PHOU(100)), 50/50 w% (PHOU(50)) and 0/100 w% (PHOU(0)). The endotoxicity was determined by the limulus amebocyte lysate test and was below 10 EU/g mclPHA. The polymers were dissolved in methylene chloride (3 w%) and cast to glass platelets. The solvent was evaporated at room temperature for 2 days. The polymer films were dried at 80°C for 1 hour.

The coated platelets were placed in 6-well plates and about 20'000 fibroblasts or HBMCs were seeded on the polymer films in proliferation medium. They were studied after 3 and 10 days under the fluorescence microscope and eventual weight losses caused by degradation were determined gravimetrically after removing the cells. Contact angles were measured with a Krüss contact angle measuring system G10.

RESULTS: Good biocompatibility could be observed for 3T3 fibroblasts and for HBMCs on the 3 PHOUs. 3T3 fibroblasts adhered well on all mclPHA films. The proliferation of HBMCs on PHOU(100) was good and the distribution uniform as shown in Figure 1. Interestingly, on PHOU(50) and PHOU(0) aggregate formation could be observed. After 10 days, no polymer loss could be measured gravimetrically.

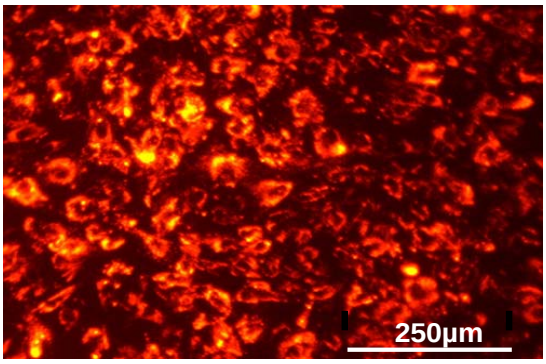

Figure 1: Fluorescently stained HBMCs on PHOU(100) after 10 days of proliferation.

Table 1. Molecular weights and water contact angles of mclPHAs.

MclPHA	M_w [kDa]	Water contact angle [°]
PHOU(100)	236	98 ± 5
PHOU(50)	285	109 ± 5
PHOU(0)	304	113 ± 5

DISCUSSION & CONCLUSIONS: The experiments show that PHOU are biocompatible to 3T3 fibroblasts and HBMCs. 3T3 fibroblasts and HBMCs proliferated well on all PHOUs although their surfaces were hydrophobic as shown in Table 1. Biodegradation of the high molecular weight PHOUs is most likely slow so that no weight loss could be measured within 10 days. Further experiments are needed to elucidate the reason for inhomogeneous cell dispersal on certain PHOUs.

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Enhanced bone regeneration by a bioresorbable and bioactive PLGA membrane

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INTRODUCTION: In Guided Bone Regeneration, a membrane is implanted to create a compartment that can easily be occupied by bone. In order to avoid a second surgery required for its removal, a bioresorbable poly (lactide-co-glycolide) membrane was developed. The intrinsic rigidity of the polymeric material was overcome by using the plasticizer NMP (N-methyl-2-pyrrolidone), which has recently been shown to be bioactive, as it enhances osteoblastic maturation in vitro as well as bone regeneration in vivo.

METHODS: In vitro, MC3T3-E1 pre-osteoblastic cells seeded onto TCPS plates were tested for early and late osteoblastic responses, namely: ALP (Alkaline phosphatase activity), osteocalcin protein secretion and Alizarin Red calcium nodule formation respectively. At the molecular level, cell extracts were analyzed by Western Blots for Smad 1,5,8 phosphorylation (related to the initiation of the BMP-2 signalling pathway), as well as mRNA levels via quantitative RTPCR (Q-PCR) for specific osteoblastic markers. (BSP:bone sialoprotein, OCN:Osteocalcin)
In vivo, PLGA membranes containing NMP were implanted into a non-critical size rabbit calvarial defect, in parallel with similar membranes without NMP, commercially available Osseoquest® e-PTFE, and empty defects as controls (n=6). 4 weeks after implantation, bone regeneration was analyzed both qualitatively and quantitatively by histology, histomorphometry as well as micro computer tomography (micro-CT) analysis.

RESULTS: NMP acting synergistically with rhBMP-2 increases ALP activity, mRNA levels of various bone related transcription factors and ECM proteins, correlating with efficient translation into osteocalcin protein secretion into the medium. Already after 5 min. of stimulation, NMP/rhBMP-2 combination also promoted the acceleration of Smad 1,5,8 phosphorylation. At later time points, an increase in calcium deposition was also measured.

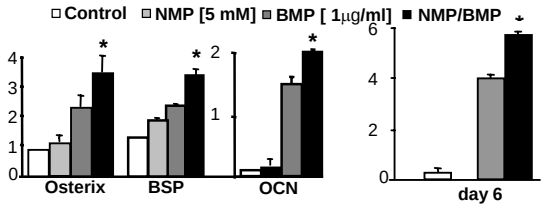

Fig. 1:Q-PCR of osterix after 24h, *BSP and OCN after 6 days of stimulation respectively (left). OCN protein concentration (ng/ml) in cell medium after 6 days of stimulation (right).*

In vivo, empty control samples failed to bridge the bone defect, while membrane-implanted defects successfully filled it with newly formed bone to different extent. The in vivo results illustrate that the guided bone regeneration membrane releasing NMP enhances bone formation significantly with respect to any of the other treatments performed.

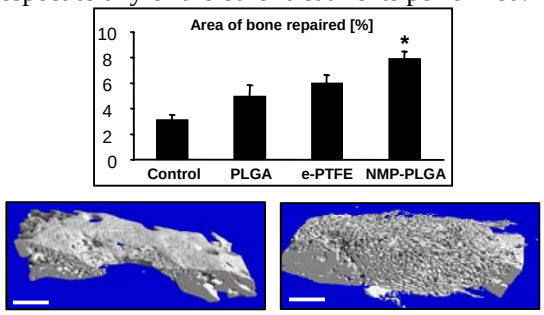

Fig.2:Histomorphometrical evaluation of the different treatments performed (top). Micro-CT cross-sections of the empty defect and NMP-PLGA membranes respectively. (Scale bar = 1mm)

DISCUSSION & CONCLUSIONS: The acceleration of Smad 1,5,8 phosphorylation, Q-PCR, expression of ALP and OCN results might indicate an interaction between NMP and the BMP pathway. The mechanism of NMP action is still unclear, however further investigations are being carried out in this respect.
This findings could be translated into novel treatment strategies for bone regeneration, where NMP improves the biological activity of autologous/exogenous BMP.

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Response of Adult Human Articular Chondrocytes to a Non-fouling RGD-peptide Modified Surface

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INTRODUCTION:

Prior to implantation to induce repair of cartilage defects [1], chondrocytes are typically expanded in tissue culture treated polystyrene (TCPS) to increase their number. However, during expansion, cells de-differentiate and their re-differentiation capacity is often limited. Since chondrocyte phenotype strongly depends on the interaction with RGD [2], here we hypothesized that a RGD-peptide modified surface (i) supports chondrocyte attachment and growth, and (ii) enhances the post-expansion ability of cells to re-differentiate and form cartilaginous tissues.

METHODS:

Adult Human Articular Chondrocytes (AHAC) from cartilage biopsies of three donors were isolated and expanded for two passages [3] on TCPS coated with RGD-functionalized PLL-*g*-PEG (*RGD*) or – as controls – on TCPS coated with non-functionalized PLL-*g*-PEG (*PEG*) [4] or on non-modified TCPS. AHAC were assessed for the capacity to attach and proliferate on the different substrates. Cell morphology was analyzed by confocal laser scanning microscopy (CLSM). The phenotype of expanded cells was determined by RT-PCR quantification of mRNA expression of collagen types I, II and X. Expanded AHAC were induced to re-differentiate by culture as 3D pellets in serum free medium containing TGFβ1. Resulting tissues were assessed histologically (Safranin O stain for glycosaminoglycans, GAG), immunohisto-chemically (stain for type II collagen), biochemically (content of GAG/DNA) and by RT-PCR [3]. Mann-Whitney tests were used to determine statistically significant differences.

RESULTS:

AHAC attached and proliferated comparably on TCPS and *RGD*, whereas attachment on *PEG* was significantly reduced (5-fold). As compared to cells expanded in TCPS, chondrocytes grown in *RGD* expressed significantly higher (5-fold) levels of collagen type II mRNA, a marker of chondrocyte differentiation, and formed more filopodia (*Fig. 1*). Expression of types I and X collagen were unchanged. Following 3D pellet culture, tissues generated by cells expanded on *RGD* contained 20% higher amounts of GAG/DNA than those expanded on TCPS. However, expression levels for type II collagen mRNA were not statistically different and only small differences were observed following tissue stain for GAG and type II collagen.

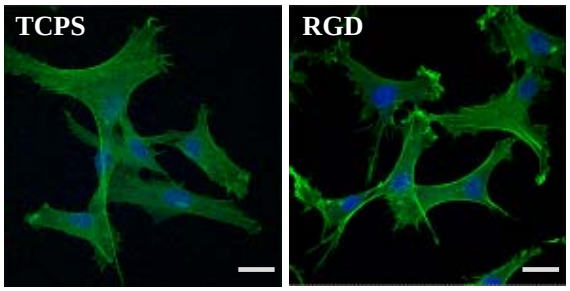

Fig. 1: CLSM images of fixed and fluorescently labeled AHAC. AHAC on RGD formed more filopodia than on TCPS. Green: actin filaments, blue: nuclei. Scale bars: 20 µm.

DISCUSSION & CONCLUSIONS: Our findings indicate that the bioligand RGD, presented using PLL-*g*-PEG chemistry, supports AHAC attachment and proliferation. In addition, chondrocytes expanded in *RGD* better maintained the differentiated phenotype, likely by specific integrin interactions. However, following transfer in a 3D environment, differences in cells expanded in the different substrates were less marked. This suggests that effects mediated by specific chondrocyte-RGD interactions are reversible, and prompts for the use of scaffolds with a *RGD*-modified surface for 3D culture of chondrocytes and engineering of cartilage grafts.

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Biomineralization of calcium phosphate

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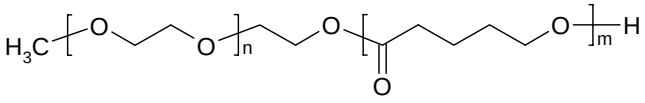
Biomineralization, as the process of controlled formation of hybrid organic-inorganic tissues in the living, is subject to a growing interest which is not to be limited to biology and tissue engineering. Generally, the engineering of inorganic precipitates by means of polymeric crystallization additives enables the synthesis of hybrid materials that are not otherwise accessible but often have useful properties.

This work, meant to be at the interface between Chemistry and Biology, combines biocompatible synthetic polymers in water with an inorganic material from the living. But since knowledge in this domain remains rather empirical, our strategy is to study systems of progressively growing complexity. Hence, our main additives for the crystallization of calcium phosphate consist in a range of synthetic diblock copolymers (polymethyloxazoline, glucose, oligo-aminoacids and genetically engineered diblocks. We aim at gaining a better understanding of the mechanisms involved in this interaction, among which we distinguish: chemistry (initiation of the crystallization by one of the blocks) and template effect (polymer self-assembly).

One of the used diblocks is Methyl-Poly(ethylene glycol)-*co*-polyvalerolactone. This Polymer can be easily prepared through a cationic ring opening polymerization of δ-Valerolactone with MPEG as a macroinitiator.



Effects of the additives were observed with X-ray crystallography, scanning electron microscopy and thermal analysis. Experimental conditions such as pH and maturation time were also varied.

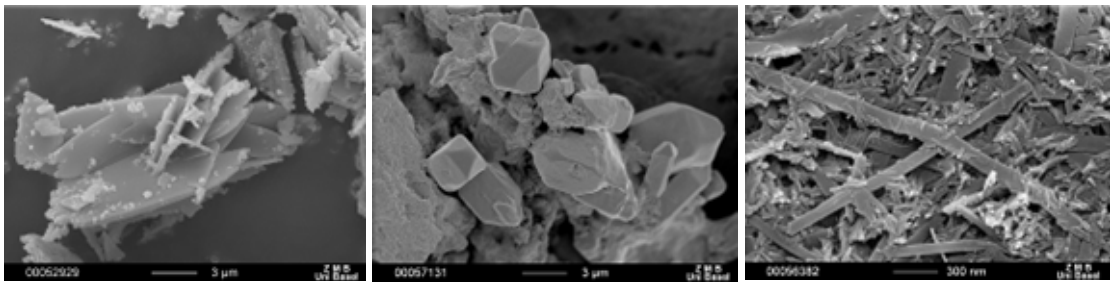


Figure 1.Brushite, hydroxyapatite, octa-calcium phosphate crystals, obtained in presence of a) no additive b) PEG-PVL c) PEG-PLGlu

(a) Water-in-water mesophases for templating inorganics, Andreas Taubert, Ernst Furrer and Wolfgang Meier, *ChemComm*, **2004**, 2170-2121

Chitosan thermosetting hydrogel for local drug delivery

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INTRODUCTION: Injectable and biodegradable drug-delivering biomaterials are of utmost importance for tissue repair. In the search for the ideal drug carrier, we selected injectable hydrogels for their ease of application and widespread use in topical delivery. Chitosan, a biopolymer extracted from crustaceans, was chosen for its unique combination of biocompatibility, biodegradability into innocuous moieties, bioadhesivity and tissue-promoting abilities [1]. The ability to produce *in situ* a gel implant can be obtained with thermosetting hydrogels that combine injectability at room temperature with an increased viscosity when injected a 37°C. This paper proposes a novel chitosan thermosetting hydrogel developed as a drug delivery platform. We characterized its rheological properties and drug-delivery abilities.

METHODS: Chitosan from Aldrich was used after purification by dissolution-precipitation and dialysis, and reacetylation up to a 50% degree [2]. Rheological measurements were carried out on a cone-plate 4°/60mm rheometer Rheostress 1 (Haake, Germany). Gel transition was defined as the intercrossing of the G' storage modulus curve with G" loss modulus. We characterized the deacetylation by RMN and molecular weight by asymmetrical field flow fractionation. *In vitro* drug release was performed in PBS buffer, at pH 7.4 and 37°C in sink conditions. Drug models were an anionic dye, erythrosine B, as well as FITC-marked proteins and antibodies (insulin, BSA, IgG).

RESULTS: High molecular weight (2 MDa) chitosan solutions were able to produce gels at 37°C following simple neutralization with sodium hydroxyde, although with very slow gelation and weak viscosity increase. Addition of selected polyols or polyoses accelerated the gelation and increased the mechanical properties of the final gel. In order to produce a stable formulation for a medical application, we tested the thermosetting properties before and after lyophilization. Addition of trehalose preserved the thermosetting properties, as shown on Fig. 1. Faster gelation can be obtained by increasing polyose concentration.

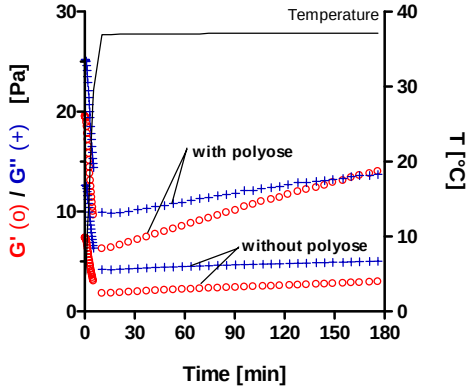

Fig. 1: Rheological behaviour of a sterile thermosetting chitosan after lyophilization and reconstitution. Polyose addition preserves thermosetting properties.

The release of anionic dye or insulin was almost complete (90% released) in two days. Larger molecules such as BSA or antibodies were released over 1 week. Previously obtained *in vitro* results also demonstrated that a growth factor, TGF-β3, kept it bioactivity when released from the chitosan gel as shown by an increased proteoglycan synthesis of chondrocytes.

DISCUSSION & CONCLUSIONS:

The use of specific polyoses preserved thermosetting behaviour following lyophilzation and reconstitution, allowing long-term storage for medical use. Advantageously, the partial chitosan reacetylation provided sufficient solubility to avoid the need for stabilizing charged molecules such as glycerophosphate that could interfere with many charged drugs. Loading and release of drugs over short time periods was shown. Taken together, these data show the feasibility of a chitosan thermosetting gel for local drug release.

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Poly(hexyl-substituted-lactides): Novel functionalized PLA for delivery of hydrophobic drugs from semi-solid polymers and PEG-Copolymer-Micelles

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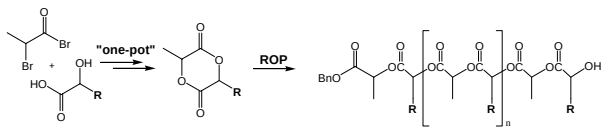
INTRODUCTION: The synthesis of new mono- and disubstituted lactides and their controlled ring-opening polymerization to biomaterials with tailored properties and functionalities optimized for hydrophobic drug delivery will be presented. The biodegradable PLA-backbone is conserved while additional side groups bespoke hydrophobicity, viscosity, degradability and drug release. Different novel polymers are considered increasing the pool of improved poly(lactides) for medical applications. In combination and copoly-merization with other monomers, e.g. standard PLA or PLGA, or also other polymeric matrices e.g. PEG, or further funtionalization of the free hydroxyl- or the deprotected carboxyl endgroup, many new drug delivery devices can be designed.

METHODS: To obtain new poly(lactide) based materials with defined properties we apply the controlled ROP of lactides and lactones, using tin (II)-2-ethylhexanoate, "tin-triflate" and DMAP, respectively, which provide new possibilities for the polymerization of the sterically more demanding and until now not successfully controlled polymerization of substituted lactides.[1,2] In a *two-step one-pot synthesis* various novel mono-substituted lactides were prepared. Their ROP lead to polymers with good controlled molecular weights and narrow polydispersities. [3]



Scheme 1: Monomer and novel functionalized PLA Synthesis

RESULTS & DISCUSSION: Novel hydrophobic PLAs were synthesized by introducing alkyl side groups (R= isopropyl, butyl, hexyl, benzyl, dimethyl). Initial studies showed here interesting material properties for the poly(monohexyl-substituted-lactides) (PmHLA). Their glass transition temperatures (T_g) and viscosities could be modulated from T_g= -42 to -10°C and 50 to 4850 Pa.s, by varying the polymer molecular weight and the number of hexyl groups along the polymer chain. Thus PmHLA is easily injectable with a standard syringe and needle and can be used in solvent-free pharmaceutical formulations. In comparison standard PLA with the same molecular weight has a T_g~+40°C! The degradation products from the in vitro degradation under physiological conditions in phosphate buffer pH=7.4 at 37°C were analyzed by ESI-MS. Hydrolysis lead first to the corresponding oligo-ester fragments and finally to the non toxic 2-hydroxy octanoic acid and lactic acid. The degradation mechanism of PmHLA is shown to be of the "bulk erosion" type and comparable to standard poly(D,L-lactide). [4] Next to these semi-solid homopolymers novel amphiphilic PEG-PHLA di-block copolymers by ROP of hexyl-substituted lactides with PEG were synthesized.[5] Micelles of sizes between 30 and 80nm were successfully prepared and an increased hydrophobic character of the micelle core with an increasing number of hexyl side groups along the polyester chain was proven by Nile Red and Griseofulvin, respectively incorporation. The low Critical Micelle Concentrations (CMC) (8-8.5 mg/L) allow to envision the use as drug carriers in very diluted conditions, e.g. in intraveneous injections. The PEG-PHLA composition and the molecular weights of the 2 segments in the copolymers can be easily fine tuned for further optimized properties of these micellar drug delivery systems. The presented technology platforms of novel alkyl-substituted PLA for medical applications are protected by a patent.

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Controlled release of tetracycline from biodegradable and biocompatible tricalciumphosphate bone substitute material

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INTRODUCTION: Local antibiotic delivery systems are promising alternatives to the systemic antibiotic treatment in the prevention of bone infections in orthopaedic surgery [1]. Therefore, we developed a tetracycline (Tet)-laden bone substitute material based on tricalciumphosphate (TCP) granules. Release kinetics were modulated by using different types of biodegradable polymers to incorporate Tet. *In vitro* evaluation showed a good biocompatibility of the device and maintenance of the stability and bioactivity of released Tet.

METHODS: TCP granules (calc-i-oss™) were coated with poly(lactide) and poly(lactide-co-glycolide) (PL(G)A) incorporating tetracycline (Tet). To modulate release kinetics six different formulations were prepared (A – F). Tet loading of TCP granules was determined by a modified HPLC method as previously described [2]. The thickness of the PL(G)A coating layer was analysed by using a scanning electron microscope. Tet release kinetics were investigated *in vitro* by incubating 100 mg of TCP granules (n = 3) in 1 ml phosphate buffer at 37°C during 67 days and Tet concentration was analysed by HPLC. A reporter cell line (CHO XM 111.10) with a Tet-responsive SEAP expression vector [3] was used to measure the biological activity of released Tet. Cellular biocompatibility testing was performed according to ISO 10993-5 guidelines using an osteoblast cell line (MG-63).

RESULTS: TCP granules contained between 22.4 and 29.2 µg Tet per mg implant material.

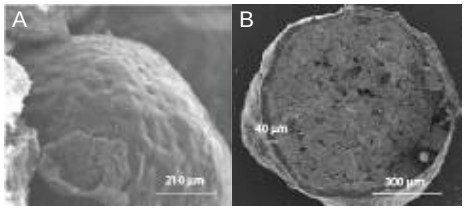

Fig. 1: Surface morphology (left) and cross section (right) of TCP composites incorporating Tet within the polymer coating layer.

In vitro release studies showed that Tet release was prolonged over a time period of 67 days and that release kinetics were dependent on the type of used polymer coating (*Fig. 2*).

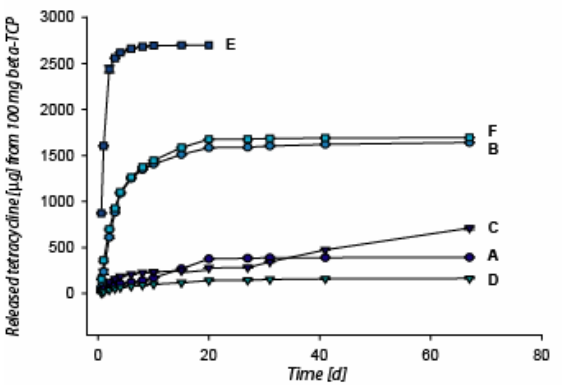

Fig. 2: In vitro tetracyline release from six different TCP composite formulations (A-F).

All formulations were able to induce a biological response as demonstrated by the reduction of SEAP expression in the genetically modified CHO cell line (*Tab. 1*). *In vitro* biocompatibility analysis showed no reduction in cellular viability.

Table 1. Alkaline phosphatase activity in response to released tetracycline from TCP granules.

	SEAP activity [µM/min] after 24 h	± SD
Negative control (without Tet)	300.0	± 22
Positive control (20 µg/ml Tet)	76.8	± 8.5
Formulation A-F	70.4 – 82.0	± 6.2

DISCUSSION & CONCLUSIONS: The developed local antibiotic delivery system allows sustained Tet release with different kinetics while providing osteoconductive properties. *In vivo* evaluation of this bone substitute material is currently under investigation.

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Image-based analysis of 3D-printed scaffolds for bone augmentation

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INTRODUCTION: Rapid prototyping technology, especially 3D printing (3DP) has become attractive for fabrication of bone augmentation scaffolds. One main advantage of 3DP is the ability to create scaffolds with controlled open porosity for osteointegration [1]. Porosity analysis on the different length scales, however, is a major challenge. In addition to the classical methods for the integral determination (intrusion, gravimetry), we used image-based pore's characterization using synchrotron-radiation-based micro computed tomography (SRµCT).

METHODS: Scaffolds (d = 3 mm, h = 6 mm) were fabricated out of hydroxyapatite (HA) granulate using an experimental 3D printer [1] with the spatial resolution of 106 dpi and the slice thickness of 200 µm. The green bodies were sintered at 1250 °C to achieve the necessary mechanical stability. SRµCT was performed at the beamline BW 2 (HASYLAB at DESY, Hamburg, Germany) in absorption contrast mode using the photon energy of 24 keV and the voxel size of 4.1 µm. 3D data were reconstructed by the filtered back-projection algorithm. The distance transform for quantitative 3D porosity characterization was obtained by the Gigatools software [3].

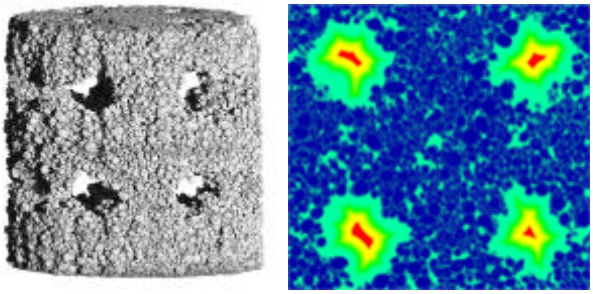

Fig. 1: 3D representation of a SRµCT tomogram of a 3D-printed HA scaffold to visualize the macro-pores realized by 3DP (left). The right image shows a distance map of one characteristic slice of the scaffold. Material is blue colored. The other colors represent distances in air to the scaffold material.

RESULTS: Fig. 2 shows the cross-section of a single HA granule. Here, the nano-porous structure is easily seen. An integral determination of this nano-porosity is obtained interpreting the histograms of the tomograms comparing dense and porous HA. The macro- and micro-porosity above the spatial resolution of the tomographic imaging is comprehensively quantified calculating the distance maps of the binarized 3D data (cp. Fig. 2B). The determination of the threshold is difficult to fix. Therefore, the mean porosity versus all possible threshold levels was analyzed, in detail.

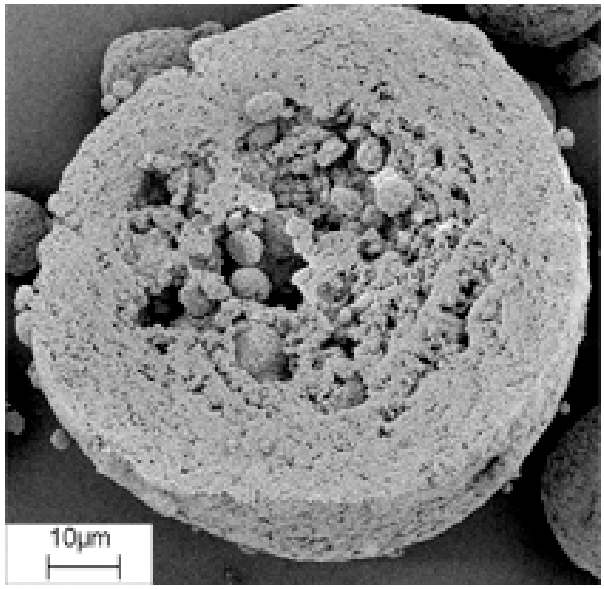

Fig. 2: SEM cross-section of HA granule with nano-pores.

DISCUSSION & CONCLUSIONS: The true micrometer resolution of SRµCT allows the precise determination of the granules shape, the pore sizes and the pore size distribution of the investigated porous scaffolds. The interconnectivity of 3D printed pore structures can be analyzed on the scale well below the cellular level. Furthermore, the determination of the intergral nano-porosity becomes possible non-destructively. This information is crucial for the optimization of the scaffold fabrication process and, finally, for the osteointegration.

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Corrosion of alkali calcium phosphate bioglasses

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INTRODUCTION: Corrosion is a common process for all glass systems even though in many cases it is scarcely perceivable due to the extremely low corrosion rate values. Calcium phosphate glasses are of interest as bioglasses, while powder samples of different compositions can be used as fertilisers. The leaching process is usually appreciated by mass loss while the release of cations is a process more specific and can be selectively controlled by the structure of glass. An additional possibility is to apply proper heat treatments for the partial crystallisation of glasses in order to develop convenient crystalline phases in which the cations are encompassed in bonds of controllable strength.

METHODS: The investigated samples belong to CaO-P_2O_5-K_2O and CaO-P_2O_5-Na_2O glass systems. They were obtained from homogenized mixtures of $CaCO_3$, $(NH_4)_2HPO_4$ and K_2CO_3 or $Na_2CO_3 \cdot 10H_2O$ analytical grade reagents by melting at 1100^{o}C, respectively 1000^{o}C, and quickly undercooling at room temperature.

The corrosion behaviour was followed at 37^{o}C in static regime by immersion of samples in different solvents simulating biological media (desalinized water, physiological serum and chlorine acid solution with pH 1.5) by measuring the mass of samples maintained in the mentioned solutions at room temperature for different times. The samples mass was determined with an accuracy of 0.1 mg. The glass surface area to solution volume ratio was around 15 m^{-1} for all samples.

RESULTS: The data obtained in the dissolution static test are expressed by the relative mass loss of samples. For the calcium-phosphate system with 10 mol % K_2O one remarks that up to 50 % substitution of P_2O_5 by CaO, the dissolution rate corresponds to the expected behaviour in the three solutions, i.e. the corrosion resistance decreases in physiological serum and chlorine acid solution, but for a higher replacement of P_2O_5 by CaO the behaviour is changed. This change is not observed for the samples containing 30 mol % Na_2O.

DISCUSSION & CONCLUSIONS: In order to explain the different corrosion behavoiur of calcium phosphate glasses in the investigated dissolution media, beside the composition of glasses and solvents is necessary to take into account the short range order characterizing the samples. The structural stability of cations in glass matrices is correlated with their local symmetry.

Pure phosphate glass consists in a continuous random network (polymeric structure) of quasi-tetrahedral PO_4 units wherein phosphorous is four coordinated and only three of the oxygen atoms of each unit bridge to neighboring units, while the forth is doubly bonded to the central phosphorous atom. The presence of the modifier like alkali and alkaline earth species decreases the number of bridging oxygens (P-O-P bridge) in PO_4 units, while its negative charge increases. The decrease of corrosion resistance is also due to the presence of the alkali ions that diminish the network consistency [1]. The initial stages of the aqueous reactions always results in the leaching of alkali and alkaline earth species from the surface of the glass to create a P_2O_5-rich surface layer. It is generally believed that in the initial stages of the leaching reaction, the contact of liquid water or vapour water with the glass surface leads to an exchange of alkali and alkaline earth ions in the glass with hydrogenated ions in the aqueous environment. (i.e. ion exchange or interdiffusion mechanism). Another mechanism proposed [2] is based on the diffusion of molecular water into the glass and its chemisorption at the non-bridging oxygen sites where alkali and alkaline earth species reside in the glass. These differences can be discussed also in relation with the cationic radii of the glass network modifiers, because calcium and sodium radii are very close each to other (1.14 Å) and well different from that of potassium (1.51 Å) and, at the same time, in relation with the cationic field strengths.

In the potassium containing samples one observes two leaching stages relative to the incipient dissolution. In the investigated sodium containing samples the highest corrosion stability was observed in physiological serum, while in decationised water and physiological serum one observes after 50 hours the occurrence of a shoulder in the release curve denoting that the glass corrosion is suppressed.

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Bone Formation on Permanent and Transient Implants

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INTRODUCTION: The surface state of an implant has substantial influence on the bone apposition and, therefore, on the functionality and stability of the implant. Calcium phosphate surface coatings (BONIT®) give rise to bioactivity of metallic implants by stimulating a rapid contact osteogenesis and thus supporting the healing process. In case of bone defects the efforts are focussed on filling the gap with a synthetic bone graft substitute the structure of which is highly similar to natural bone. The bone graft substitute BONITmatrix® mainly composed of a highly porous calcium phosphate network not only fulfils this requirement but also completely degrades during reossification until the new bone has replaced the graft. Both biomaterials stimulate the body to form new bone and are fully resorbed during healing. The structural properties and the resorption behavior of these osteoinductive biomaterials are discussed as well as their mode of action in vivo.

METHODS: Structural characterization was performed by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) of surfaces and microtome slices, respectively. The composition was analyzed by energy dispersive X-ray spectroscopy (EDX) and atomic emission spectroscopy (AES). The solubility of the BONIT® coating was studied at 37 °C in 0.05M Tris-HCl-buffer (pH=7.3) [1], the cumulative release of calcium from BONITmatrix® was investigated in the same buffer.

RESULTS & DISCUSSION: Fig. 1 shows a SEM micrograph of the BONIT® coating on the surface of an implant (left). The alignment of the BONIT® crystal platelets nearly perpendicular to the surface enhances ingrowth of new bone into the porous implant surface and provides excellent capillarity, i.e. high capability for the immobilization of growth factors from the blood. The coating is mainly composed of brushite with small additions of hydroxyapatite. Chemically BONIT® represents a compound that is very similar to pre-stages of bony calcium phosphates during mineralization. The BONIT® coating increases the bone to implant contact area (Fig. 1, right) and is completely resorbed by the body within 6 - 8 weeks.

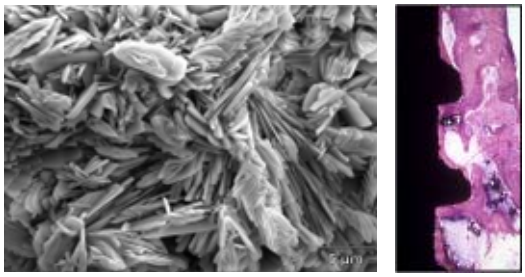

Fig. 1: BONIT® morphology (left) and new bone apposition on a BONIT® coated dental implant (pink stained, right).

In Fig. 2, the blue colored regions indicate a rapid multicentrical formation of new bone within the interconnecting pores of the degradable bone graft substitute BONITmatrix®.

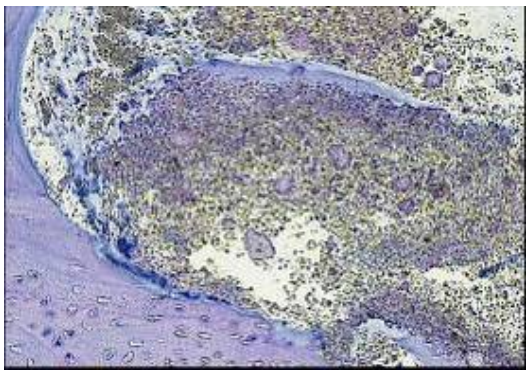

Fig. 2: Bone formation (blue) in BONITmatrix® (after 4 months)

BONITmatrix®consists of a porous network of hydroxyapatite and tricalcium phosphate (weight ratio: 60/40) glued with a silica xerogel. The biomaterial has a high porosity of 60% ± 5% due to interconnecting nano- and micro-pores which produce an internal surface of 100 m^2/g [2]. It induces a rapid reossification of the defect whereas simultaneously the material gradually degrades. After 8 months the biomaterial is completely replaced by new autologous bone.

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Ultrasound assisted osseous fixation of degradable polymer implants

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INTRODUCTION: Ultrasound assisted osseous fixation of degradable polymer based implants **(**BoneWelding® technology) is an innovative, new method for bonding dental or orthopaedic implants directly to bone. This process employs ultrasonic energy to liquefy a polymeric interface between implants and the host bone. Polymer penetrates the pores of the surrounding bone, rapidly solidifies and forms a strong and uniform microscopic inter-digitation interface between implant and bone. The project combined in vivo and in vitro experimental studies with computer simulations to fully characterize the ultrasonic fixation process.

METHODS: Quasi-static and dynamic biomechanical testing in a variety of bone analog materials (Sawbone, solid rigid and cellular rigid 7.5 to 20pcf) was used to fully characterize the mechanical performance of the inter-digitation interface. For testing, injection molded polymer dowels (length 25mm, Ø 3.5mm, PLDLA 70/30) were used, insertion was achieved using ultrasonic power at 20kHz and with an amplitude of about 50µm. Titanium cancellous bone screws (Ø 3.5mm) were used as reference, details are given elsewhere [1]. A large animal (sheep), *in vivo* study was conducted to evaluate short and long term (2 – 6 months, 3 sheep per time point, implantation in distal femur and proximal tibia) biological response to the process and implant materials.

RESULTS: The microscopic inter-digitation interface was found to be stronger than the surrounding host material – failure always occurred in the bone analog material. Consequently, substantially superior mechanical performance of the ultrasonically inserted dowels in comparison to conventional bone screws was demonstrated (Figure 1). This has also been confirmed in highly osseo-porotic human bone (proximal humerus) [2] .

In the *in vivo* study no inflammation or excessive fibrous tissue formation was observed. No bone necrosis was found in the interface zone. Active bone remodeling was seen around the implant. A decreased rate of bone resorption immediately adjacent to the implant in comparison to control sites distant from the implant was observed.

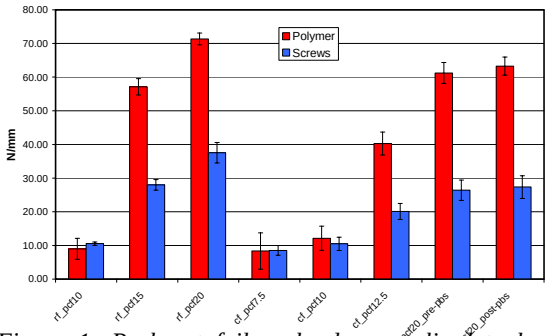

Figure 1: Push-out failure load, normalized to bone depth. Ultrasonically inserted dowels consistently outperformed conventional metallic screws.

Micro finite element computer models were developed to compare the local mechanics of load transfer through the microscopic inter-digitation interface and conventional bone screws. Peak bone stresses were lower for the ultrasonically inserted implants. Conventional screws produced point loading of individual trabeculae with significantly higher stress levels.

DISCUSSION & CONCLUSIONS: Ultrasound assisted fixation of polymeric implants has shown to be a biocompatible and mechanically superior fixation technique if compared to anchorage capacity of cancellous bone screws. These findings have has also been confirmed in highly osseo-porotic human bone (proximal humerus) comparing the ultrasonically inserted polymeric implant with a titanium suture anchor [2]. Furthermore, the ultrasound specific torque-free axial insertion process will allow non-circular implant cross-sections that will display distinct mechanical advantages. In 2005, the technology has been clinical introduced for maxillo-facial surgery.

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Activation and biomechanical assessment of an injectable hybrid osteoconductive – osteogenic bone substitute

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INTRODUCTION: Current research is focusing on injectable osteoconductive materials. Injectable CaP cements offer a minimal-invasive use, but lack osteogenic properties. In order to create a combined osteoconductive / osteogenic bone substitute we used in this study a synthetic, injectable and resorbable Brushite / ß-tricalcium-phosphate (β-TCP) scaffold (chronOS Inject) and impregnated it with a transglutaminase (plasmatransglutaminase – F XIII). We evaluated the activity of the osteogenic protein and the biomechanics of the mixture.

METHODS: Activation study: In order to evaluate the reaction of the osteogenic protein F XIII to the fluid phase of chronOS Inject (sodium hyaluronate), different pH solutions (pH 4 – 7), sodium hyaluronate and chronOS Inject were mixed with F XIII and the protein activity and F XIII release was detected with ELISA.

Biomechanical study: The injectability of the chronOS Inject / F XIII mixture was assessed measuring the force required to inject the mixture through a 1 ml standard syringe without a cannula. 3 repeats were preformed with and without F XIII.

RESULTS: Activation study: F XIII is not influenced by low pH and the sodium hyaluronate. The release of F XIII from chronOS Inject could not be measured due to clouding of the liquid.

Biomechanical study: We saw an increase of the injection force in the presence of F XIII which increased with time after mixture.

DISCUSSION & CONCLUSIONS: F XIII is not affected by the liquid phase of chronOS Inject, which therefore may be a good carrier for the osteogenic protein. In a further test we could remove the clouding by filtration, however to what extend the amount of protein was also reduced needs to be further investigated. Furthermore the osteogenic protein affects the injectability of the cement which may be due to the fact, that the sodium hyaluronate and the protein increase cement viscosity or a change of the liquid / powder ratio. The injectability needs to be optimized testing various liquid / powder ratios in the future in order to produce a hybrid injectable bone substitute.

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Effect of α-tricalcium phosphate milling on the incorporation of calcium sulfate dihydrate during cement setting

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INTRODUCTION: Apatite cements harden very slowly. Thus, efforts have been devoted to increase their reactivity, e.g. by means of milling or use of additives. Despite these efforts, there is still little understanding of the kinetics of cement setting. Ideally, a cement should not react for a few minutes, and then harden rapidly. The goal of this study was to assess the effect of a setting retarder (citrate ions) in an α-tricalcium phosphate (α-TCP) based cement containing two setting accelerators, Ca-sulphate dihydrate (CSD) and phosphate ions. Additionally, α-TCP powders milled for various durations were used.

METHODS: α-TCP powder was milled for 15, 75, 150 and 225 min. Particle diameters and specific surface area (SSA) of the resulting powders varied between 7.1–11.9 μm, and 1.93–2.28 m^2/g, respectively. Cements were prepared by mixing 4 g of powder (3.63 g α-TCP, 0.37 g CSD) with 1.72 ml of $C_6H_5Na_3O_7 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 2H_2O$ 0.2 M solution. The citrate concentrations ranged from 0.00 to 0.10 M. The paste was then transferred to a syringe whose tip has been cut off. Fifteen minutes after setting, the samples were placed in 0.15M pH 7.4 phosphate buffer solution at 37ºC. After 6, 24 or 48 hours of incubation, they were taken out and dried at 37 ºC. Finally, the samples were processed to obtain cylinders of 12 mm in diameter.

Characterizations included: setting time, compressive strength, SSA, microstructure (SEM), and composition (XRD and XRF). Additionally, the ability of the blocks to be machined was evaluated.

RESULTS: The setting time of the cement increased almost exponentially with an increase of the citrate concentration. Interestingly, no significant change of the cement setting time was observed between 0.00 and 0.01 M concentrations or between the α-TCP powders milled for 75, 150 and 225 min. The powder milled for 15 min had significantly longer setting times. These results suggest that the presence of an amorphous phase does not modify the initial reactivity (expressed by setting time measurements). The cement SSA increased with an increase of the citrate concentration, but this effect was limited to about 10%. No difference of SSA was detected between 6, 24 and 48 hours of incubation. The ability of the cement blocks to sustain mechanical machining varied significantly according to the milling time: in the 36 samples prepared with powders milled for 15 and 75min, only one sample broke during machining. On the other hand, only 12 out of 36 samples produced with α-TCP milled for 150 and 225 min remained intact. Therefore, not all samples could be tested for compressive strength. Values were close to 30 MPa, i.e. close to previously-reported results. The citrate concentration and α-TCP milling time did not significantly affect the results.

An increase of α-TCP milling time reduced the time until setting completion and decreased the CSD content in the hardened cement. As XRF does not show any difference of sulphur content in the samples and no traces of Ca-sulphate hemihydrate or anhydrite were found in XRD data, the disappearance of CSD may originate from incorporation of sulphate into the apatite structure, or from formation of an amorphous phase.

SEM pictures did not reveal any effect of citrate ions on the cement microstructure, contrary to α-TCP milling time: longer milling time transformed a plate-like structure into a tube-like one with more open and globular microstructures (Fig 1).

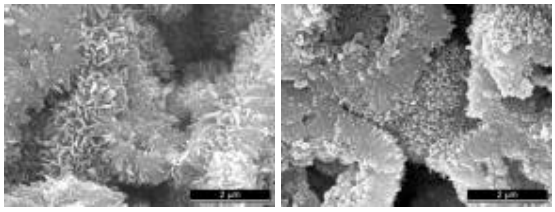

Fig. 1: SEM microstructure; milling time: left: 15min; right: 225min.

DISCUSSION & CONCLUSIONS: Prolonged α-TCP milling (i) does not shorten the cement setting time, (ii) reduces the time until setting completion, (iii) leads to a disappearance of crystalline CSD, and (iv) results in very brittle cement samples.

Wax Up Failures in the Removable Partial Dentures Technology Using Light Curing Materials

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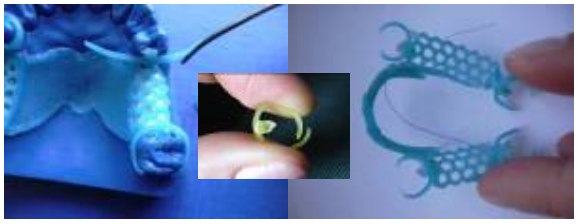
INTRODUCTION: The wax patterns of the metallic frameworks of the removable partial dentures could be made directly on the cast, using profiled waxes like: „Ti-Light" or „LiWa" (light curing „waxes'). This study intends to describe the problems, failures of this new technology.

METHODS: The study was made at the Department of Removable Partial Dentures Technology, Specialization Dental Technology, University School of Dentistry, Timisoara, Romania, between 2004 and 2006, on 30 casts with different edentation types [4]. The wax patterns of removable partial dentures metallic frameworks were made directly on the cast using profiled waxes like:„Ti-Light"(Ti Research GbR, Mainbernheim, Germany) and „LiWa"(WP Dental GmbH Bevern/Hamburg).

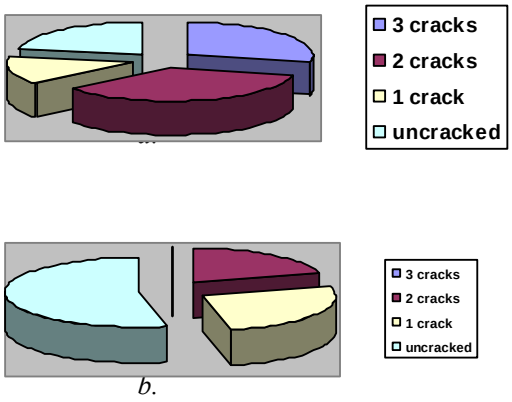
RESULTS: Light-curing waxes are sticky (to casts and instruments) and difficult to use (Fig. 1a). Therefore they need precision in profiles applying. Even the wax patterns seem to have a great elasticity (Fig. 1b), their removing from the cast have to be made with great patience, not producing materials cracks or fractures (Fig 1c, d, 2a, b).



a. *b.*

c. *d.*

Fig. 1. Light curing wax up:a. LiWa – sticky to instruments, b. elasticity testing for both materials, c. cracks al Ti-Light, d. cracks at LiWa.



b.

Fig. 2. Statistics of cracks: a. for Ti-Light, b. for LiWa.

DISCUSSION & CONCLUSIONS:

1. Using light curing waxes is a novelty in the field of removable partial dentures technology [1,2,3,4].
2. Reducing the working time and economizing some materials used for intermediary stages are major qualities that will impose this materials in practice.
3. Even if not all of these biomaterials details are known, the system could be completed, that will modify the working system in the field of removable partial dentures technology.

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Glass and Bioactive Glass Nanopowders by Flame Synthesis

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INTRODUCTION: Glasses and glass ceramics consist of undercooled, partially of fully amorphous materials and are used since thousands of years for their intriguing properties. Certain glass compositions known as Bioglass® or bioactive glasses were found to form a tight bond with living bone [1]. This materials can be applied for bone repair and regeneration of defects arising from trauma, tumour and osteoporosis [2]. Solid state reactions and sol-gel processes can be limiting in terms of composition, high remaining solvent content and generally require an annealing or sintering step after preparation [3]. Here we would like to show the direct synthesis of bioglass nanoparticles in a high temperature environment and illustrate the advantages in terms of accessible composition, homogeneity and particle size.

METHODS: Precursors of all materials have been prepared by correspondingly mixing 2-ethylhexanoic acid salts of calcium and sodium with hexamethyldisiloxane tributyl phosphate and fluorbenzene. The liquid mixtures were fed through a capillary into a methane/oxygen flame. Oxygen was used to disperse the liquid leaving the capillary (Fig. 1).

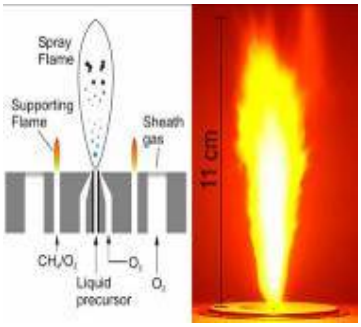

Fig. 1: (a) Sketch of the experimental set-up. (b) The burning spray of a calcium phosphate producing flame synthesis unit.

RESULTS: Flame spay synthesis was used to prepare 45S5, 45S5F and 77S-type of Bioglass. The as-prepared bioactive glasses consist of XRD-amorphous nanoparticles with a primary particle size of 20-80 nm (SSA_{BET} 70-200m^2/g depending on composition).

After immersion of the 45S5 type of bioactive glass in simulated body fluid (SBF) for 7 days, Raman spectroscopy and electron microcopy was used to assess the surface of the material. The formation of a crystalline carbonated apatite layer was observed thus confirming the bioactivity of the material (Fig. 2).

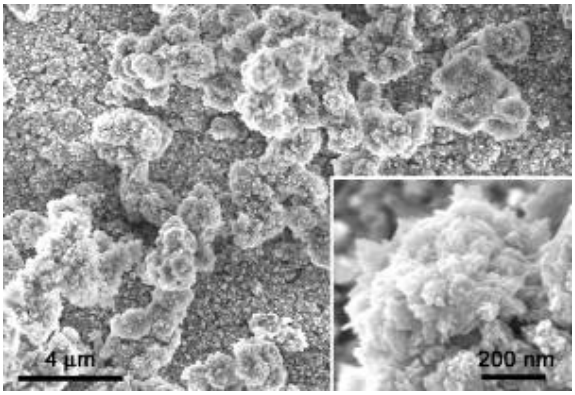

Fig. 2: SEM image of Bioglass 45S5 after incubation in SBF.

DISCUSSION & CONCLUSIONS: Complex glasses consisting of 5 elements could be readiliy produced as nanoparticles using a flame spray reactor. The high temperature synthesis allows the preparation of a well defined, homogenous and amorphous material. The material showed deposition of bone-like carbonated hydroxyapatite after immersion in SBF, which corroborates the bioactivity of the bioactive glass.

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Structure and chemical stability of yttrium silica sol-gel microspheres

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INTRODUCTION: Biodegradable silica microspheres incorporating yttrium were investigated for possible use in the radio therapeutic treatment of special cancer tumors. The microspheres are designed to carry the radiation inside the cancer site, in order to provide a high and localized dose of beta radiation.

Sol-gel method allows at relative low temperatures obtaining materials of high purity and homogeneity with a controlled rate of biodegradability. By spray-drying method, microspheres with desired size can be obtained. Several yttrium silica microsphere batches were prepared in order to optimise the processing parameters. *In vitro* testing was focused on cations dissolution from microspheres immersed in Tris buffer solution at 37^{o}C for up to 27 days. Structural characterization of the materials was done before and after immersion in the used solution.

METHODS: Silica microspheres of less than 50 μm of diameter containing yttrium were prepared by sol-gel and spray- drying methods. The obtained microspheres were thermal treated in order to control the biodegradation rate. The incorporation of yttrium in the microspheres was analyzed by Back Scattered Electron Imaging of Scanning Electron Microscope (BEI-SEM) equipped with Energy Dispersive X-ray Analysis (EDX). The chemical stability of the yttrium silicate microspheres have been investigated under in vitro conditions using Tris buffer solution with pH= 7.4, for up to 27 days. The yttrium and silica release behavior in the solution was analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and UV-VIS spectrophotometer. The structural changes occurred during the immersion time have been evidenced by Fourier Transformed Infrared Spectroscopy (FT-IR).

RESULTS: BEI-SEM/EDXA analysis showed that yttrium was well and homogenously encapsulated in some batches of the silica microspheres. The biodegradation results indicate that the silica release significantly decreased with the thermal treatment applied. The solution analysis points out a small release of yttrium from the thermal treated samples in the immersion solution. The yttrium release depends not only on the thermal history of the samples but also on the incorporation of yttrium in the silica network.

The main vibrational modes of the Si-O-Si groups have been identified from the FT-IR spectra of the samples. The spectra exhibit a large band comprised between 1000 and 1200 cm^{-1} which, together with the 796 cm^{-1} band could be assigned to the asymmetric and symmetric stretching vibration mode of the Si-O-Si groups. The band located around 464 cm^{-1} is attributed to Si-O-Si bending vibration. The stretching vibration of the Si–OH and NO^{3-} groups, located around 943 cm^{-1} and 1385 cm^{-1} disappear after the thermal treatment applied. The FT-IR spectra of the reacted microspheres revealed the structural changes occurred in the silica network during the immersion time. These changes depend on the type of the microspheres, indicating the influence of the processing conditions on the samples' structure and on the dissolution behavior of the microspheres in solution.

DISCUSSION & CONCLUSIONS: The biodegradation results reveal that the stability of the microspheres in the used solution depends on the thermal treatment applied as well as the incorporation of the yttrium in the silica network. The incorporation of yttrium in the microspheres strongly depends on the processing parameters. Using the optimal processing parameters yttrium can be well incorporated and stabilized in the silica microspheres, supporting the idea of using the sol-gel microspheres in radio therapeutic applications.

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Microstructure and bioactivity of bone cements for prosthetic surgery

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INTRODUCTION: Porous materials have been used in surgical implant design to fabricate devices or augment soft or hard tissues, as coatings on prostheses to accommodate tissue ingrowth for biological fixation and as scaffolds to facilitate the regeneration of tissue. Polymer-ceramic composite are widely used in orthopaedics as suture materials and fixation devices due to their biocompatibility-ability to support bony growth (osteoconductive) and also bone bioactive (to form a calcium fosfat layer on its surface) [1]. Our approach is to compare the microstructure, bioactivity and biocompatibility of two different types of biocomposites: BIOLOS 3 and ANTIBIOTIC SIMPLEX (containing erythromycin and colistin) from in vitro study in simulated body fluid.

METHODS: Electrochemical measurement were performed using Na^+ and Ca^{++} selective electrodes for different time intervals, during 34 days incubation in SBF. The study is focused on microscopic (SEM) analysis of the graded layer structure before and after immersion in SBF, as the development of an active layer is expected [2]. The FTIR spectra of the grown hydroxyapatite layer have been recorded after different times intervals in the range 500-3000 cm^{-1} and compared with those of the native materials. Hemolysis tests and osmotic fragility of red blood cells were performed, as the blood compatibility is dictated by the manner in wich the material surface interact with blood constituents (red blood cells, platelets, proteins) [3]. Spectrophotometric measurement were performed at 540 nm to quantify the hemoglobin released in order to estimate the extent of red cell lysis. The absorbance of blood samples (following exposure to both biomaterials) were normalised with that of control blood.

RESULTS AND DISCUSSION: A considerable diminution of Ca^{2++} has been obtained in SBF after 14 days incubation, for both types of biocomposites, the Na^+ concentration being less affected during the times (fig.1). FTIR measurements showed typical vibration modes of P-O stretching vibration at 987 cm^{-1} and C-O asymetric bending from CO_3^{2-} at 812 cm^{-1}, wich can be used to estimate the relative layer thickness using the Buger-Lambert-Beer' law for the optical density at these wavelenghts. The SEM images shows regular, cuasi-spherical aggregates with an average size of 10 μm, grouped homogeneously, the densisy of the aggregates being greater for the ANTIBIOTIC SIMPLEX (fig.2). During the hemolysis tests, the blood sample exposed to BIOLOS 3 exhibited the maximum value of absorbance, suggesting the maximum lysis of red cells.

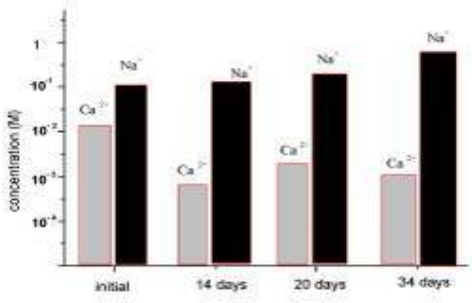

Fig.1: Effect of Simulated Body Fluid incubation on Ca^{2+} and Na^+ concentration, after different time intervals.

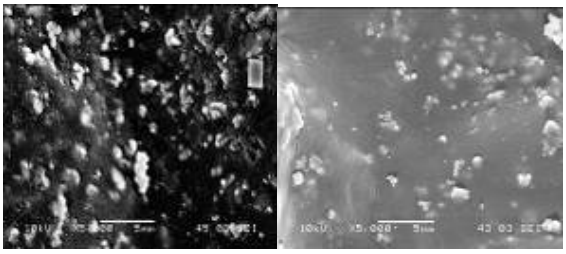

Fig.2: Morphology of hydroxyapatite crystals after 14 days incubation at ANTIBIOTIC SIMPLEX surface(left) compared to BIOLOS 3(right) in the same conditions.

CONCLUSIONS: Upon hemolysis tests and osmotic fragility of red blood cells and comparation of the results obtained with the above techniques, one can suggests that ANTIBIOTIC SIMPLEX is a better material then BIOLOS 3.

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A new preparation technique of the resorbable silicon-substituted calcium phosphates

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INTRODUCTION: It is well established that trace elements such as magnesium, carbon (as carbonate) and fluorine (as fluoride) in calcium phosphate ceramics and coatings strongly influence both the biological response of implant materials, and affect the crystallographic, mechanical and chemical properties of manufactured ceramics and coatings. Since recently, silicon is also included into this list of trace elements: silicon-contained and/or silica-substituted calcium phosphates catch the attention of researchers and industrial manufacturers of bone grafts.[1,2] Precipitation is the standard preparation technique of silicon-substituted calcium phosphates which occurs by mixing of calcium- and phosphate-containing solutions at pH ~ 10 in presence of a silicon-containing compound, followed by aging, filtration, washing off, drying and sintering at 900 – 1200 °C. In this report we are going to present a simplified preparation technique of silicon-substituted calcium phosphates.

METHODS: Chemically pure solid calcium nitrate $Ca(NO_3)_2 \cdot 6H_2O$, solid ammonium dihydrogenphosphate $NH_4H_2PO_4$, liquid ammonium hydroxide (25% aqueous solution), and a very fine dry powder of silica-gel were used as initial chemicals. In order to prepare silicon-contained calcium phosphates, solid calcium nitrate was mixed with solid ammonium dihydrogenphosphate on the desired Ca/P molar ratios. Then, a necessary amount of solid silica-gel (the amount depended on the desired Si/P molar ratio) was added and the entire blend was mixed. Afterward, the mixture was placed into a alumina crucible and a small amount (to get approximately 1 : 1 of the liquid/solid ratio) of either pure deionized water or aqueous solution of ammonium hydroxide was added into the crucible. Finally, the crucible was placed into a furnace and the suspension was sintered at 1000 °C for 4 hours (ramp rate 3°C/min), followed by cooling down to ambient temperature. The resulting powder was analyzed by SEM, XRD, IR-spectroscopy and by chemical analysis.

RESULTS: The experimental results revealed that depending on the initially selected Ca/P and Si/P molar ratios of the initial reagents, the final compounds might have either the structure and composition of a silicon-substituted hydroxyapatite, similar to that described by Gibson et al.[2], or a triphase mixture of silicon-stabilized α-tricalcium phosphate, hydroxyapatite and small amount of β-tricalcium phosphate similar to the product described by Langstaff et al.[1]. Dissolution experiments performed in slightly acidified (pH = 6.5) solutions of simulated body fluid revealed dissolution of both compounds.

DISCUSSION & CONCLUSIONS: Therefore, resorbable silicon-contained calcium phosphates might be easily prepared by a single stage which makes unnecessary the stages of aging, filtration, washing and drying. Presumably, the biological behavior of the simplify-prepared silicon-containing calcium phosphates should not be different from those prepared using the routine way (this needs to be verified). However, we believe that this simplified preparation technique of silicon-substituted calcium phosphates will reduce the production costs and make artificial bone grafts more affordable in the market.

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Design and Characterization of 3D-Printed Hydroxyapatite Scaffolds using Synchrotron-Radiation-based Micro Computed Tomography

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INTRODUCTION: To reconstruct the bony tissue within large defects, highly porous biocompatible scaffolds based on hydroxyapatite granulates can serve as 3D templates for initial cell attachment and subsequent tissue formation. The design of the scaffolds has to fulfill different criteria to ensure cell viability and function. These include nanoporosity to allow diffusion of molecules for nutrition and signaling, micropores to ensure cell migration and capillary formation as well as macropores for arteries and veins. Synchrotron-radiation-based micro computed tomography (SRμCT) is a unique, non-destructive technique to characterize the scaffolds with respect to the integral nanoporosity and the detailed morphology of micro- and macropores under in-vitro conditions. Comparing different scaffold designs seeded with progenitor cells, appropriate preparation procedures for the cell-scaffold composites can be uncovered.

METHODS: Three different scaffold structures of cylindrical shape (D = 4 mm, H = 6 mm), shown in Fig. 1, were realized using 3D printing [1]. Multipotent ectomesenchymal progenitor cells isolated from human tooth were cultured in DMEM containing 10% FCS. Subsequently, each scaffold was statically loaded with 80 µl cell suspension containing 2×10^5 cells and incubated for 2 h at 37 °C. The cell-scaffold constructs were harvested after 28 days and fixed in 4% paraformaldehyde for 24 h. The SRμCT measurements were carried out in absorption contrast mode at the beamline BW 2 (HASYLAB at DESY, Hamburg, Germany) using the photon energy of 24.5 keV. The pixel length corresponds to 3.9 µm and the spatial resolution determined by the modulation transfer function to 6.5 µm [2].

RESULTS: Similar to the *compacta*, the 3 designs exhibit a denser outer structure to provide the mechanical stability. As demonstrated in Figs. 1 and 2, a central channel provides space for larger blood vessels and medium flow. Perpendicular to this main channel, 50-100 channels with diameters between 300 and 400 µm allow cell migration. The rounded structures offer a relatively large surface for cell attachment. Finally, the designs ensure that the total X-ray absorption is comparable in all directions perpendicular to the main channel axis.

The SRμCT reveals that the scaffolds are built out of nanoporous granulates forming interconnected microchannels.

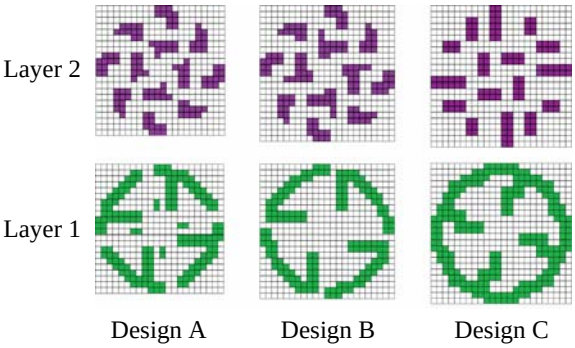

Fig. 1: The scaffolds are formed by alternating layers. One pixel corresponds to 240 µm.

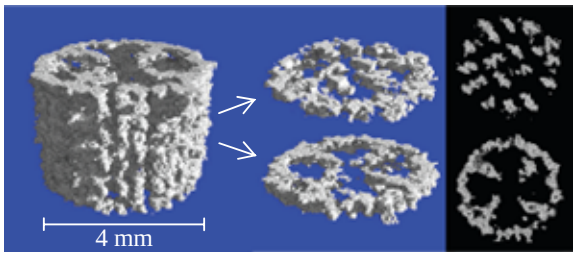

Fig. 2: 3D representation of SRμCT tomogram of design C. Extracted slices with a height of 240 µm are given on the right.

DISCUSSION & CONCLUSIONS: SRμCT allows non-destructively analyzing the porosity of ceramic scaffolds from millimeter to nanometer scale. Therefore, the method provides complementary information to classical histology, avoiding any kinds of preparation artifacts due to sectioning. Due to mechanical stability and the larger number of channels, design C is favored.

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The microleakage in open-sandwich class II restorations

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INTRODUCTION: In order to overcome the inherent composites disadvantages such as the polymerisation shrinkage and the weaker adhesion at the composite-dentin interfaces several solutions were proposed. The step-by-step incremental technique, transparent matrices, reflecting wedges and improved adhesive systems solved only partially these problems. A slightly different approach was proposed - the open-sandwich technique - which consists in a cervical layer of another class of material prior to composite insertion in class II cavities [1]. The aim of the present study is the to assess the microleakage between different restorative materials and the dentine cervical margin, as well as between these materials and a composite used in the open-sandwich technique in class II cavities.

METHODS: Fifty noncarious and crack-free mandibular third molars were used. The teeth were randomly divided into five groups each containing ten teeth. Class II box-like cavities without occlusal retention cavity and any mechanical retention means were prepared on each tooth using a rounded fissure diamond bur. The gingival margin was located 1 mm below the cemento-enamel junction. The bucco-lingual width was 4 mm and the mesio-distal depth of the cervical margin 1,6 mm. The prepared cavities were filled as follows: group no. 1 (AM) - bonded amalgam (Amalcap Plus -Vivadent with Scotchbond MP Plus adhesive system - 3M Espe) + composite, no. 2 (GI) - glass ionomer (Ionofil Molar - Voco) + composite, no. 3 (RMGI) - resin modified glass ionomer (Vitremer - 3M Espe) + composite, no. 4 (COMP) - compomer (Compoglass - Vivadent) + composite, no. 5 (FC) - full composite filling using the step-by-step incremental technique. The composite used was Spectrum TPH (Dentsply DeTrey). The cervical increment in the open-sandwich technique as well as the first composite increment for the composite fillings was 2 mm thick. All specimens were stored for 7 days at 37 ^{O}C in demineralised water. All specimens were thermocycled 500 cycles between 5-55 ^{O}C and stored 24 hours in methylene blue 10%. All specimens were sectioned along a mesio-distal plane through the middle of the cervical margin. To assess dye penetration, the cervical areas of the sections were examined using an optical microscope. At the cervical interface the microleakage scores were chosen as follows: 0 - no penetration, 1 - penetration up to 1/2 of the depth of the cervical margin, 2- penetration more than 1/2 of the depth of the cervical margin, 3 - penetration along the axial wall. For groups no. 1-4 microleakage between the cervical material and composite (the junction interface) was also assessed: 0 - no penetration, 1 - penetration up to 1/2 of the interface, 2 - penetration more than 1/2 of the interface, 3 - penetration along the axial wall. The obtained scores were analysed with Kruskal-Wallis an Mann-Whitney U tests.

RESULTS: For the cervical interface Kruskal-Wallis test showed statistically significant differencens among groups ($p<0.001$, $\alpha=0.001$). The median values were AM - 0.5, GI - 3, RMGI - 0, COMP - 1, FC -1.5. The Mann-Whitney U test diplayed significant differences between AM-GI ($p<0.001$, $\alpha=0.001$), AM-FC ($p=0.0022$, $\alpha=0.01$), GI-RMGI ($p<0.001$, $\alpha=0.001$), GI-COMP ($p<0.001$, $\alpha=0.001$), GI-FC ($p=0.0016$, $\alpha=0.01$), RMGI-FC ($p<0.001$, $\alpha=0.001$). For the junction interface Kruskal-Wallis test showed statistically significant differences among groups ($p=0.0177$, $\alpha=0.05$). The median values were AM - 0.5, GI - 0, RMGI - 0, COMP - 0. The Mann-Whitney U test displayed significant differences between AM-RMGI ($p<0.05$, $\alpha=0.0118$), AM-COMP ($p<0.05$, $\alpha=0.0118$).

DISCUSSION & CONCLUSIONS: The least microleakage at the cervical interfaces was obtained using AM or RMGI, while GI exhibited the contrary. At the junction interfaces the least microleakage was obtained using RMGI, COMP or GI, while AM gave higher microleakage values. Overall, the best adaptation at cervical and junction interfaces was obtained using the resin modified glass-ionomer - composite open-sandwich technique.

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BONE DEGRADATION DUE TO THE TENSIONS GENERATED BY FIXED PARTIAL PROSTHESIS. A THREE-DIMENSIONAL PHOTOELASTIC APPROACH.

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INTRODUCTION: Photoelastic stress analysis is based on the property of some transparent materials to exhibit colorful patterns when viewed in polarized light. These patterns occur as the result of alteration of the polarized light by the internal stresses into two waves that travel at different velocities. These patterns are formed as the result of alteration of the polarized light by the internal stresses developed in two waves with different velocities. This model, made from a transparent material capable of exhibiting a photoelastic response, is subjected to loads representative for functionally applied forces. The stresses that develop in the model as the result of the applied loads can then be visualized by examining the model with polarizing filters.

METHODS: The aim of this study is to identify the tensions that appear in the prosthetic field while inserting a fixed partial prosthesis. The dispersions of these tensions in the remaining teeth and also in the width of the maxillary bone of the edentation area are also considered. The three dimensional photo elastic methods were used. The materials used in the study were DINOX 010P resin. The actual photo elastic study was made using a horizontal polariscop. For this experiment we used a titanium fixed partial bridge put it on a maxillary model made from photoelastic material. The occlusal loading was made by a dedicated devices. After the fixed partial bridge was loaded the whole assembly was put in an oven for a termic treatment. During this treatment the load was maintained on the titanium fixed partial bridge by the dedicated device mentioned above. After the termic treatment, the maxillary model was cut into three slices in the zone of interest. The cutting direction followed a mesial-distal plane. Each slice was analyzed in order to obtain the tension dispersion in that part of the bone.

RESULTS: The results of this study are the tensions that were registered in the three space coordinates according to which the real tensions of the prosthetic filed structure were calculated. For the photoelastic analysis we used the horizontal polariscop. The dispersion tensions in the bone images were uploaded in a dedicated soft (SIGMA SCAN PRO). This soft allows to evaluate the real tensions in every point of interest from the maxillary model.

Fig. 1: Effect of stress in the bone after the occlusal loading on titanium fixed partial bridge.

DISCUSSION & CONCLUSIONS: The conclusion of this study is that the tension experimentally determined on the photo elastic model corresponds to the prototype's one. The photo elastic method is one of the election methods in determining the real tensions obtained in the investigated structure. The overloading of the fixed partial bridge will lead to periodontium trauma and to bone resorbtion, as this method indicated.

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Controlled Release of Glial Cell Line-derived Neurotrophic Factor from Biodegradable Nerve Conduits

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INTRODUCTION: Growth factors are promising candidates to improve functional outcome upon peripheral nerve regeneration. However, little is known about the impact of different release kinetics on nerve regeneration.

We therefore developed a biodegradable, multi-ply delivery system with adjustable release kinetics for glial cell line-derived neurotrophic factor (GDNF). A porous supporting hollow cylinder made of collagen was coated with concentric layers of release-modifying polymer (PLGA), in-between which GDNF was embedded. Changes in the stacking sequence and type of polymer (lactide to glycolide ratio, molecular weight and free versus esterified end groups) allowed us to tailor different release kinetics.

METHODS: Nerve conduits (NC) were produced by spinning mandrel technology. A suspension of insoluble collagen (2.5 % w/w, Avitene from Davol, Cranston, RI, USA) was deposited onto a spinning steel mandrel from a syringe. The solvent was evaporated under a laminar airflow, and the resulting tube was cut into 6 mm long NC. Various types of PLGA layers were sprayed onto the NC with an ultrasonic spray nozzle (Resomer RG 503, RG 503 H, RG 752, RG 755, from Boehringer-Ingelheim, Ingelheim, Germany; the first two digits refer to the amount of lactide (75 vs 50 %), and the third one to the intrinsic viscosity). All polymers are end-group capped, except for 503 H. GDNF in buffer (1.2 µg per NC) was deposited on top of the first PLGA layer in the central part of the NC. *In vitro* release was measured by incubating the NC in citrate buffer (pH 5, 150 mM NaCl, 0.05% Tween 20) at 37°C. Buffer exchange was daily, and GDNF was assayed by ELISA.

RESULTS: GDNF release kinetics could be efficiently controlled by selecting appropriate PLGA types (Fig. 1). NC coated with PLGA 75:25 types (NC-C and NC-D) released GDNF, after the initial burst, at lower rates than the NC coated with the more hydrophilic PLGA 50:50 types (NC-A and NC-B). Interestingly, the end-group uncapped PLGA 503 H (NC-A) mediated a slower GDNF release than the less hydrophilic end-group capped PLGA 503 (NC-B).

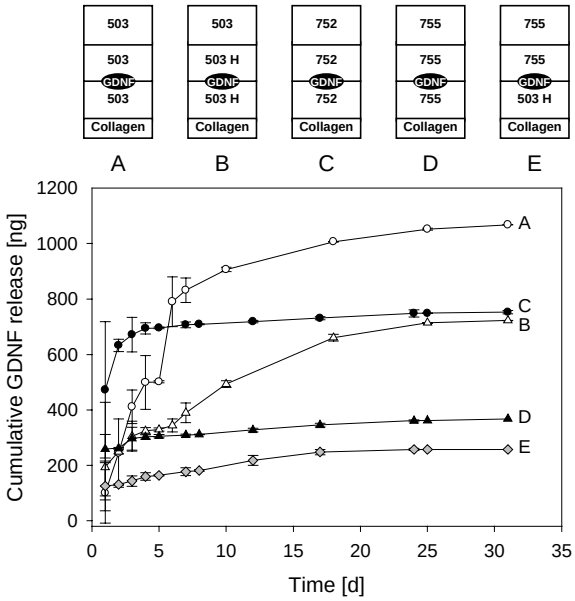

Fig. 1: Effect of polymer type on release kinetics of GDNF. The top panel illustrates the various NC construct (PLGA types are denoted by the code of the corresponding Resomers). The dimensions shown do not reflect the real proportions of the layers. The wall of the collagen tube was about 10 times thicker than the total thickness of all PLGA layers together. The bottom panel shows the in vitro GDNF release curves obtained with the different NC constructs. Means with standard deviation (n=4).

DISCUSSION & CONCLUSIONS: We developed biodegradable, multi-ply nerve conduits with adjustable release kinetics that permit control of GNDF delivery kinetics in the low ng/day range for several weeks. Changes in polymer type and coating thickness resulted in distinctly different release kinetics, although incomplete release of some formulations also revealed stability issues of GDNF. Employing NC with different release kinetics in models of peripheral nerve regeneration *in vivo*, we expect to gain insights into the optimal delivery regimen for GDNF and finally improve functional outcome after traumatic nerve injury.

ACKNOWLEDGEMENTS: The authors acknowledge financial support by GEBERT RÜF FOUNDATION (CH-Basel), SUVA (CH-Lucerne) and ETH Zurich, and thank for the kind supply of GDNF by Amgen (Thousand Oaks, CA, USA).

Tailor – made biocompatible, biodegradable bacterial polyesters

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INTRODUCTION: Poly[(R)-3-hydroxyalkanoates] (PHAs) (Fig.1) are biocompatible and biodegradable biopolyesters.[1)] Because many bacteria can accumulate these polyesters as carbon and energy storage compounds they are widespread in nature. Until now more than 100 different types with various functionalities in the side chain have been described.[2)]

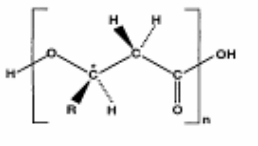

Fig. 1: PHAs are divided into short-chain-length (scl: R=1or 2) and medium-chain-lenght (mcl: R=3-12) polyesters and consist of enatiomeric pure monomers.

METHODS: All the different polyesters we isolated during this study were produced by *P. putida* GPo1 (ATTC 29347) in continuous (chemostat, D=0.1h^{-1}) cultures in either three or ten liter laboratory bioreactors. To achieve a tailor-made, exactly reproducible composition of the polymers we used double (carbon and nitrogen) nutrient limited culture conditions[3)] and different fatty acids as precursors for the desired polyesters. During the cultivation the cells were harvested and freeze-dried. Afterwards the polymer was extracted with green chemistry and further purified to remove all bacterially derived impurities. The monomeric composition of the polyesters was determined by GC, thermal properties were analyzed by DSC and the molecular weights were obtained from GPC measurements (Table 1).

RESULTS: We were able to produce a set of tailor-made poly(3-hydroxyalkanoates) from octanoic and 10-undecenoic acid. The chosen way of continuous fermentation under double nutrient limited growth conditions allowed us to produce amounts up to 1kg PHA of a defined, reproducible and endotoxin-free (<0.5EU/g) quality. (Fig. 2)

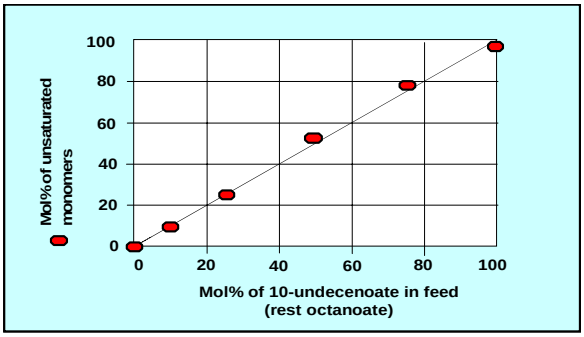

Fig. 2: The ratio of the precursor fatty acid mixture is reflected in the polymer composition.

Table 1. Physical properties of unsaturated tailor made poly(3-hydroxyalkanoates).[4]

Physical parameter	Feed composition of octanoate/10-undecenoate [mol%/mol%]					
	100/0	90/10	75/25	50/50	25/75	0/100
Tm [°C]	58.1	50.8	44.5	39.9	-	-
Tg [°C]	-33.1	-35.9	-39.5	-44.6	-47.4	-49.3
Mw [kDa]	286	251	253	290	278	290
Mn [kDa]	118	132	113	156	118	122
Mw/Mn	2.4	1.9	2.2	1.9	2.4	2.4

DISCUSSION & CONCLUSIONS: More than 100 different polyhydroxyalkanoates have been described in literature until now. None of them, except PHB, PHB/HV and PHB/HHx are available in sufficient amounts for further testing and research. With our production method we are opening the field to study biocompatibility and biodegradation of novel PHAs wich will lead to new developments and applications of this very interesting class of biopolyesters in medicine and industry.

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Injectable and PLGA coated β-TCP granules hardening in situ: an in vitro study

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INTRODUCTION: Bone fillers on the basis of calcium phosphate are widely used in bone tissue engineering [1]. Health professionals demand bone grafts, which can be injected directly into a bone defect. The preservation of the graft's integrity over a certain period of time is a key issue. To provide an injectable and in situ hardening biomaterial, β-TCP granules coated with poly(lactide-co-glycolide) PLGA [2] were mixed with the plasticizing agent N-Methyl-pyrrolidone NMP which makes the granules stick together. In this study, the in vitro behaviour of this biocomposite is reported with regard to the release of degradation products.

METHODS: β-TCP granules with diameter range 500-1000µm coated with PLGA were mixed with NMP to give an injectable material. Using a mould simulating a canine tooth, 11 samples were produced, each of which made with approx. 165mg granules. The in vitro study was conducted for each sample in 10ml phosphate buffered saline at pH 7.4 and 37°C. Degradation was observed by measuring pH value, the release of calcium ions, D- and L-lactate after 1, 2, 7, 14 and 21 days. In each solution, calcium concentration was determined with atomic absorption spectroscopy, D- and L-lactates were determined by using a kit from r-Biopharm (D). The results of the injectable material were compared to the results obtained with PLGA coated granules fused by heat at 80°C (RootReplica™).

RESULTS: Fig. 1 shows the release of D- and L-lactate over 21 days. The release from the injectable granules is higher due to the partial dissolution of the PLGA coating when mixing the granules with NMP. In contrast, the coating of the RootReplica granules remains more stable until approx. day 15 where standard deviation of both material groups overlap. pH value decreases within the test period from 7.4 to approx. 6.1 for both materials due to release of lactic acid, the injectable granules showing always a slightly lower pH value. Structural integrity of the samples made with the plasticizer was lower after the study period compared to the samples with heat-fused granules. Fig. 2 shows the cumulative Ca^{2+} release over 21 days, what corresponds to the degradation behavior found in Fig. 1.

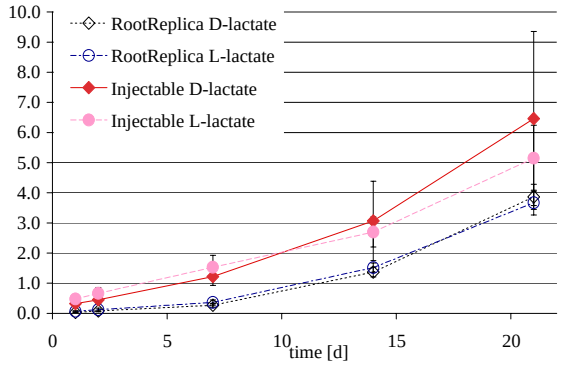

Fig. 1: cumulative D- and L-lactate release of injectable granules and RootReplica granules.

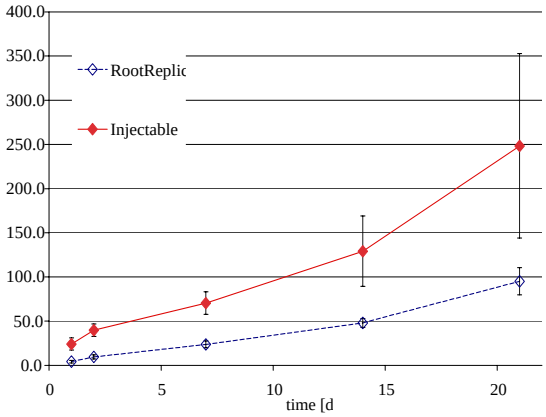

Fig. 2: cumulative Ca^{2+} release of injectable granules and RootReplica granules.

DISCUSSION & CONCLUSIONS: The injectable granules degraded faster than the heat-fused granules within the period of 21 days. A modification of the plasticizing agent must be seeked to optimize the degradation with respect to an extended stability of the implant. Nevertheless, the injectable version formed a stable implant in situ, which represents an advantage for the use as bone graft material. The application of the new biocomposite is easy and thus, it may be an interesting material for dental and orthopedic applications.

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ACKNOWLEDGEMENTS: The authors thank the staff of DS and ZZMK, notably Mrs. B.Sener and Mrs. R.Späni.

MICROLEAKAGE ASSOCIATED WITH THE USE OF CERAMIC INSERTS

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INTRODUCTION: One of the major clinical disadvantages associated with the use of composite resins in the direct technique for class II cavities is the gap formation in the tooth-filling interface [1,2]. In class II cavities, this lack of adaptation of the material to tooth structure particularly occurs at the gingival margin [3,4]. In order to overcome the undesirable effects of consecutive microleakage, recent restorative techniques followed three main directions: incremental insertion, placing a polymerization tip inside the composite interproximally and the placement of glass or ceramic inserts inside the proximal of proximo-occlusal restorations. Since their introduction, glass and ceramic inserts have improved significantly. The most precise systems are those which use calibrated burs: Cerafil (Komet), Cerana (Nordiska Dental) and β-Quartz (Lee Pharmaceuticals). The Sonicsys approx system (Vivadent and Kavo) uses sonic driven diamond tips for the final cavity preparation. These diamond tips are mounted in the Sonicflex 2000L/N handpiece (Kavo). The inserts are fabricated from leucit-reinforced glass ceramic and their size (2, 3 or 4) fits to the corresponding preparation tips. According to manufacturer's indications, the inserts are luted in the cavities using a flowable composite resin, namely Tetric Flow (Vivadent). With this system, class II cavities can be restored in a consistent and cost-efficient manner. Marginal adaptation in class II cavities restored using the Sonicsys approx ceramic inserts was investigated by several authors [3,4]. Their results prove that a perfect adaptation at the gingival margin located in enamel can not be achieved

METHODS: Forty noncarious and crack-free mandibular third molars were used. The teeth were randomly divided into to four groups each containing ten teeth. One mesial box cavity was prepared on each tooth, using the diamond tip Sonicsys approx no.3. The cervical margin of each cavity was located about 1 mm coronally to the cementoenamel junction. The prepared cavities were filled using Sonicsys approx ceramic inserts no.3 and four resin-based materials: group 1 – Tetric Flow; group 2 – Admira Flow; group 3 – Nexus 2; group 4 – X-flow. After finishing and polishing, all specimens were stored in distilled water for 7 days at 37ºC, thermocycled 1000 cycles between 5-55ºC, and stored 24 hours in basic fuchsine 2%. All specimens were then sectioned along a mesial-distal plane through the middle of the cervical margin. To assess the dye penetration, the cervical areas of the sections were examined using an optical microscope. The registered scores were analysed with Kruskal-Wallis and Mann-Whitney U tests.

RESULTS: Kruskal-Wallis test showed statistically significant differences among groups ($p = 0.009$, $\alpha = 0.01$). The Mann-Whitney U test displayed significant differences between Admira Flow group and Tetric Flow ($p = 0.011$, $\alpha = 0.05$), Nexus 2 ($p = 0.001$, $\alpha = 0.01$), and X – flow ($p = 0.004$, $\alpha = 0.01$), respectively.

DISCUSSION & CONCLUSIONS: The extent of microleakage in the cervical area (enamel) of class II cavities filled with Sonicsys approx ceramic inserts, depends on the material used for luting. The highest leakage occurred when Admira flow ormocer was used.

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Laser Welding Procedures Applied to Removable Partial Dentures Frameworks Repairs

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INTRODUCTION: One of the modern methods of removable partial dentures defect repairs uses the pulsed laser with relative low average outpower. This is known as a precise and rapid joining method, but its succes depends on the control of many parameters [1,2,3]. The purpose of the study was to make experimental investigations regarding the optimal welding parameters applied to Co-Cr alloys related to the framework defect.

METHODS: In order to evaluate the welding parameters several framework defects were simulated for the experimental study. The removable partial dentures frameworks were cast using a Co-Cr alloy (Vaskut Kohászati KFT, Budapest, Hungary) and as filling material a special 0.5 diameter Co-Cr wire Finalloy (Fino, Bad Bocklet, Deutschland) was used. Several cracks were simulated in different components of Co-Cr frameworks (continuous clasp, lingual bar, clasp arm). Depending on the defect type and dimension, the Nd:YAG laser (TRUMPF HL 124P LCU) was used both for joining with or without filling material, build-up welding and for a combination between the two procedures. All the repaires were made manually under argon shielding atmosphere. For preliminary attempts the peak power and pulse duration were varied, the pulse repetition rate was kept constant at 1 Hz in order to maintain a constant movement and a pulse overlapping of 80-90%. The diameter of the laser spot was maintained at 0.6 mm in all cases.

RESULTS: The welding parameters were determined for each defect type (Fig. 1,2,3) and working step (fixing, joining, filling, planing). Adequate combination of pulse energy (6-14 J), pulse duration (10-20 ms) and peak power (600-900 W) depending on the working stage improves the succes of the welding procedure.

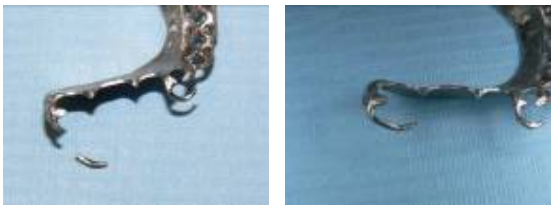

Fig. 1. Repair of a broken circumferential clasp.

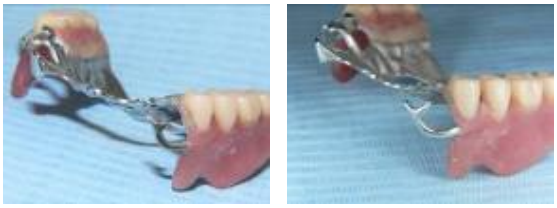

Fig. 2. Cast defect repair of a Roach clasp.

Fig. 3.Repairs of cracked continuous clasp and lingual bar.

DISCUSSION & CONCLUSIONS: The limitations of the study are in connection with the acces lack of the laser beam in the defect area or with the large defects, that can't be made directly. They need a previous casting of the missing part, followed by a welding to the framework.
Selecting the adequate combination of pulse energy, pulse duration and peak power for each welding step is necessary for the succes of the welding procedure.

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Evaluation of biological tissue response of β-TCP bone substitute in high molecular sodium hyaluronate matrix in sheep for improved handling properties

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Introduction:

Autograft bone is generally considered to be the most successful bone filling material because of its osteoconductive, osteoinductive and osteogenic properties [1]. Drawbacks of autograft use are increase of operation time and co-morbidity following the bone harvesting at the iliac crest. To encounter this problems, synthetic bone substitutes have become more and more popular in the last 20 years [1]. Particularly porous beta tricalcium phosphate (β-TCP) is a very promising osteoconductive, ceramic bone substitute. β-TCP ceramics are available in either granules or preforms. To enable surgeons an easier defect filling with β-TCP, chronOS granules have been embedded into a high molecular sodium hyaluronate matrix to form a kneadable putty, a β-TCP putty.
The biological tissue response to the bone substitute β-TCP putty, was evaluated as a function of time following implantation in bone of sheep and compared to the local tissue response to medically accepted material chronOS granules or a sham defect [2].

Methods:

The sheep study was performed at Harlan, Bioservice For Science, in accordance with the principles of Good Laboratory Practice. Three implantation periods, lasting 3, 6 and 12 weeks were employed. In each sheep, the test (β-TCP Putty) and reference (chronOS granules) device were implanted into cylindrical defects of 4 x 12 mm in both tibiae and a sham operation (no material) was performed [2].
During the implantation periods, clinical observations, measurements of body weight and evaluation of implantation sites were performed. After each implantation period, the designated animals were sacrificed. The implantation areas were explanted, bone samples were prepared and preserved for histopathological examination.

Results / Discussion:

After the 3-week implantation period, the materials chronOS granules and β-TCP Putty supported bone formation due to their osteoconductive nature. Newly built bone tissue was outgoing from the margins of the wound. Frequently, absorption or degradation of β-TCP particles by macrophages were observed [3].
At the end of the 6 week implantation period, there was a closed bone bridge between both margins of the wounds after implantation of chronOS granules and β-TCP Putty. Mostly, the individual granules or particles were completely imbedded within new bone tissue. Following the sham procedure, the wounds were closed in most samples [3].
After the 12 week implantation period, the formerly drilled holes filled with either chronOS granules or β-TCP Putty were completely closed and the sham sites healed up completely [3].

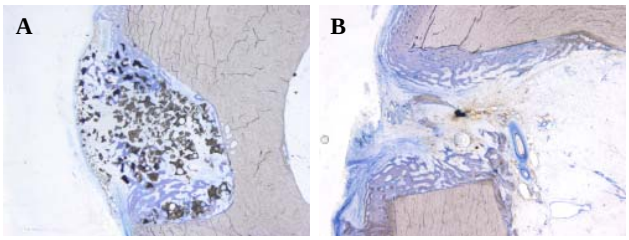

Fig. 1: Comparison of a defect filled with β-TCP Putty (A) and the sham defect (B) after 3 weeks of implantation.

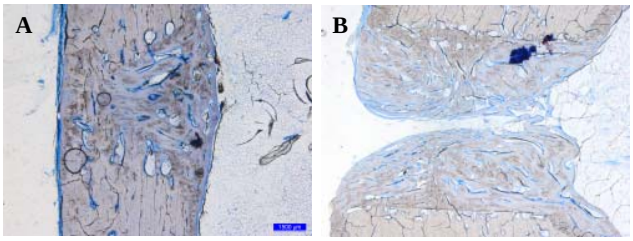

Fig. 2: Comparison of a defect filled with β-TCP Putty (A) and the sham defect (B) after 6 weeks of implantation.

Conclusions:

In conclusion, β-TCP appeared to be a bone replacement material with optimal biocompatibility, resorption characteristics and bone conduction properties for the clinical use. The addition of high molecular sodium hyaluronate to chronOS granules did not influence the local tolerance as well as the osteoconductive properties of the material, however, significantly improved the handling properties of chronOS granules.

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TEETH PREPARATIONS DEGRADATION DUE TO THE TENSIONS GENERATED BY ADHESIVE FIXED PARTIAL PROSTHESIS ON THE PROSTHETIC FIELD. A NUMERICAL SIMULATION APPROACH.

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INTRODUCTION: The basic concept of this technique is the visualization of the actual structure, which is a continuum, as an assemblage of a finite number of discrete structural elements connected at a finite number of points. The finite elements are formed by figuratively cutting the original structure into segments. Each element retains the mechanical characteristics of the original structure by specifying the appropriate mechanical properties. Additionally, a numbering system is required to identify the elements and their connecting points, called nodes, and a coordinate system must be established to identify uniquely the location of the nodal points. A large number of simultaneous linear equations are computer generated, which establishes compatibility within each element.

METHODS: The considered study required the establishing of three-dimensional model of an upper central incisor and a canine. The fixed partial adhesive prosthesis replaces the lateral missing incisor. The modeling was made by manually constructing a three dimensional model. This method implies quite a long time to generate the model and good skills in three-dimensional objects generating softs. The modeling was made using GEOSTAR, a tool of the COSMOS numeric simulation program. Two types of investigations were imposed by the complexity of the dental model. After the identification of the interest zone of the numeric dental model, it was done a numerical simulation concerning the zones mentioned before in order to increase the accuracy of the solution. These zones appear to be located between the dental tissue and the adhesive fixed partial denture. Specific biomechanical condition were imposed to this three dimensional dental model, in which the intermediary dental tooth is not represented because of the target of this study. It was considered that the adhesive fixed dental prosthesis was made by titanium, and for the dental tissues were considered the normal characteristics mentioned in dedicated literatures.

RESULTS: The computer completed the problem processing and the numerical solution revealed tension zones in the adhesive fixed dental structure, as well as separated in the dental prosthesis and in the dental tissues. For the interface between adhesive dental prosthesis and the dental tissues it was compared the maximal tension recorded (σ_{max}) with the admissible one (σ_a), the last one being specific to a dental material (titanium). If $\sigma_{max} > \sigma_a$, than an additional support is recommended.

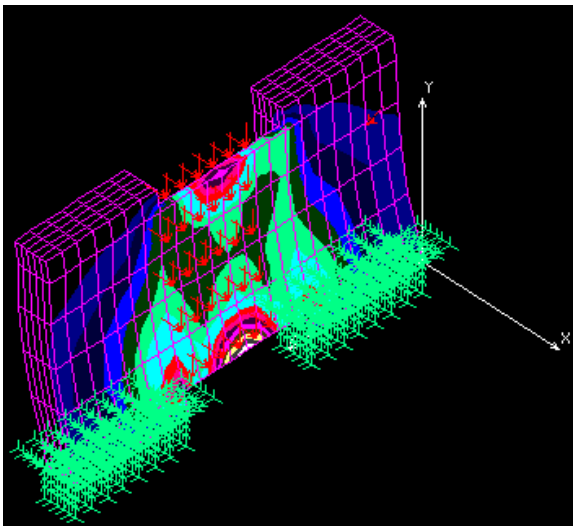

Fig. 1: Effect of stress in the teth after the occlusal loading on adhesive titanium fixed partial bridge.

DISCUSSION & CONCLUSIONS: Making an adhesive fixed dental prosthesis with an admissible tension with vales situated near the one corresponding to the dental tissues may lead to fracture of the prosthesis. The use of dental alloys (such as titanium) may be the cause for the eventual fracture of the dental support or for the debonding of the dental prosthesis.

REFERENCES: [1]C.P.Fernandes, P.O.Glantz K.Nilner (2003).- On the accuracy of some in vitro models for mechanical studies of maxillary removable partial dentures in *Dental Materials* (Volume 19, Issue 2).

FIXED PARTIAL BRIDGE DEGRADATION DUE TO THE TENSIONS GENERATED BY OCCLUSAL LOADING. A NUMERICAL SIMULATION APPROACH.

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INTRODUCTION: The finite element stress analysis technique models actual continuous structures with discrete-element mathematical representations. This approach transforms the problem into one of matrix algebra, which may be solved with the aid of a digital computer. This technique has some very distinct advantages, among which is the ability to obtain accurately the stresses throughout the structure under consideration. Further, the inclusion of any type of anisotropy (directional characteristics) and in homogeneity is conceptually possible by inserting the appropriate distribution of materials properties at the nodes of the elements

METHODS: The fixed partial dental bridge (Fig. 1) was modellated using the MODELA device. This device is mechanical modeling one. The models were obtained in the wireframe mode and also in the render mode. The model was uploaded in PROENGINEER in order to definite the margins and the specifics zone for the bridge. After that the model was imported in ANSYS in order to perform the numerical simulation. We used titanium, all ceramic system, all polymeric system, Co-Cr alloy and gold as the materials for this bridge. It was perform another set of numerical investigations for the situation of different temperature (-10 ^{0}C and 50 ^{0}C).

Fig. 1: Fixed partial bridge used for modelling.

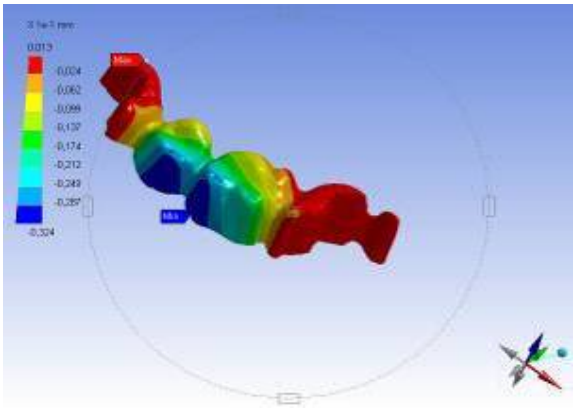

Fig. 2: Effect of stress in the fixed partial bridge after the colossal loading.

DISCUSSION & CONCLUSIONS: The conclusion of this study is that the tension numerically determined on the fixed partial bridge model are bigger in the -10 ^{0}C case, for all the materials used in this numerical simulation. The maximum deformation was found in the gold fixed partial bridge, and the minimum deformation corresponded to the Co-Cr fixed partial bridge. There were problems about all ceramic system and all polymeric system because the temperature. The decrease of the temperature leads in these cases to a fracture probability, especially in the cervical zone of the fixed partial bridge (Fig. 2).

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A Prototype System for Testing Biomaterials Properties in Controlled Cutting Conditions

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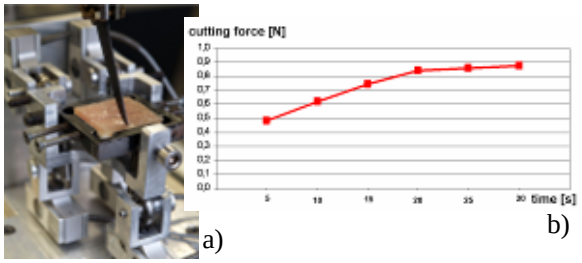
INTRODUCTION: A system for running cutting tests with blades (i.e. surgical scalpels) in controlled conditions has been developed. Cutting motion and forces are computer-controlled, reaction forces and blade-material friction are recorded during the test; moreover, the 3D microtopography of the cut surfaces can be acquired and analyzed. The system can be used to study properties and behaviour of either living tissue or natural or synthetic biomaterials to be subjected to blade cutting, for application in biomedical and surgical fields.

METHODS: The system architecture is composed of two main units: the first (cutting unit) is devoted to the execution of the cutting test in controlled conditions and to the recording of cutting forces and friction; the second (surface analysis unit) is devoted to 3D microtopography analysis of the cut surfaces.
The cutting unit is comprised of: interface for mounting different kinds of blades at various incidence angles; interface for holding specimens; unit for generating the cutting motion with real-time control of speed rates and normal cutting loads; equipment for replicating dry and wet cutting; equipment for measuring forces and friction coefficient during the test; software environment for processing and analysis of measured data, similar work can be found in [1].
The surface analysis unit is comprised of: subsystem for the acquisition of the 3D microtopography of the cut surfaces, equipped with contact sensor (stylus) or optical laser sensor, with submicrometric horizontal and vertical resolutions; software environment for the quantitative analysis of the 3D microtopography reconstructed from measured data [2].

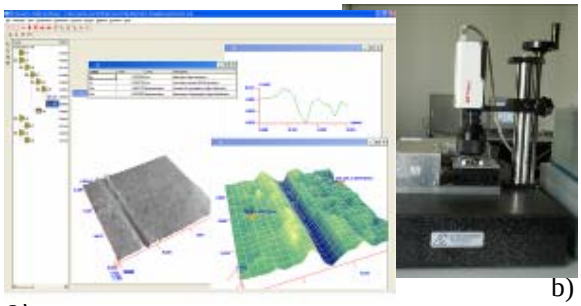
RESULTS: Cutting tests were performed on living tissue (dried pig skin) and polymeric biomaterial (PMMA) with different types of surgical scalpels. Test parameters: cut length 10 mm; cutting speed rate 25 mm/s; constant vertical load 4 N. Tangential and normal forces were recorded and analyzed (Fig.1). The cut surfaces were acquired with the 3D microtopograpy acquisition subsystem equipped with the laser sensor. (Fig. 2.a) and analyzed via software (Fig. 2.b).

DISCUSSION & CONCLUSIONS: The results of the cutting tests performed with the prototype on living tissue and polymeric biomaterial show that the developed system can be successfully used to analyze the properties of different materials as they are subjected to cutting with blades. The system can provide aid to the design and development of innovative biomaterials aimed to exhibit specific behaviour when subjected to blade cutting.



a) b)

Fig. 1: Cutting unit; a) detail of the test in progress (ceramic scalpel cutting dried pig skin); b) cutting normal load as measured by the cutting unit during the test .



a) b)

Fig.2: Surface analysis unit; a) analysis of the 3D microtopography reconstructed from measured data; b) acquisition of 3D microtopography of a PMMA specimen.

REFERENCES: [1]G. Tholey, T. Chanthasopeephan, T. Hu, J.P. Desai and A. Lau (2003), *Measuring grasping and cutting forces for reality-based haptic modeling* International Congress Series **1256**, pp 794-800. [2]R. Groppetti, N. Senin and A.Deiro (2003) *A contribution to the development of three-dimensional surface nano and micro-topography measurement and analysis techniques and systems* Proc. of the International Metrology Congress, Toulon, France.

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Influence of cross-linking process on rheology of the sodium hyaluronate matrix

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INTRODUCTION: Sodium hyaluronate (NaHA) is a common biopolymer used as medical device for many purposes. This biopolymer is a naturally occurring polysaccharide concentrated into the skin, eyes, joints… For viscosupplementation and filling applications NaHA is provided as a cross-linked matrix in order to obtain a long lasting effect. Even if the cross-linker is the same, the process of cross-linking plays an important role on the physico-chemical properties of the resulting injectable gel. The different marketed gels are studied rheologically.

METHODS: The different marketed gels are studied with an AR1000, TAinstruments, with a plate-plate 40 mm geometry, a 1000 µm gap and a solvent trap at 25°C. The analysis consists in the linear visco-elastic domain determination, a frequency sweep and a flow experiment. The comparison of the resulting rheograms allows proposing an explanation of the process.

RESULTS: The process of cross-linking provides different properties of the resulting gels. The mains products on the market are dedicated to visosupplementation of the knee joints or wrinkles filling. The major products are biphasic (particles in a fluid carrier) or monophasic (continuous phase of cross-linked chains). The monophasic products can be monodensified or polydensified. The rheology is in accordance with the structure. The monophasic gels show lower elastic modulus (G') than biphasic products with a longer linear visco-elastic domain (Fig.1). The polydensified matrix has the particularity to present a lower G' (easier to inject and to place in the tissue) with a similar linear visco-elastic domain (same mechanical resistance).

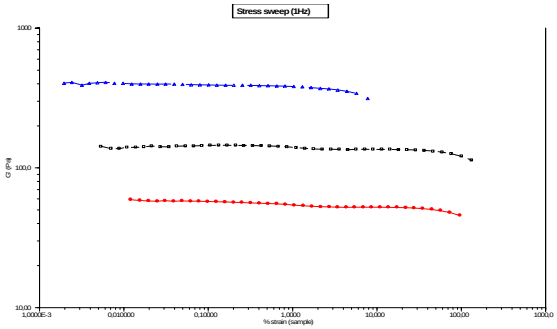

Fig. 1: Effect of the structure onto the linear visco-elastic domain (△ : biphasic product, □: monophasic monodensified product, ● : monophasic polydensified product).

The frequency sweep experiment shows a decrease of the tanδ with the decrease of the frequency in accordance with the structure (Fig.2). The more the value of tanδ is small the more the rheological behaviour of the matrix is solid elastic related to the denser cross-linked network.

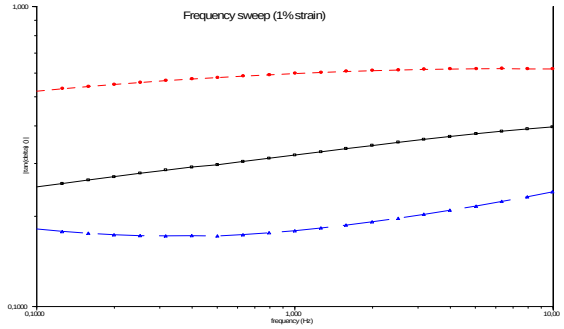

Fig. 1: Effect of the structure onto the tanδ for frequency sweep (△ : biphasic product, □: monophasic monodensified product, ● : monophasic polydensified product).

DISCUSSION & CONCLUSIONS: The rheological behaviour of various gels based on cross-linked sodium hyaluronate was studied and showed significant differences. Their biological comportment in vivo has to be analysed thanks to these results. A high rheological parameters product should induce a biological response, which is prohibited for injectable medical devices. A perfect bio-integration of the polydensified monophasic gel and the non-recognition of the implant into the body is explained by the low G' in the range of frequency and strain. The efficiency of this specific product is equal to the other and is proved by the similar linear viscoelastic domain.

ACKNOWLEDGEMENTS: This work was completed thanks to the kind help of Dr. P.Micheels who provided the samples.

Ultrasound Monitoring of the Setting of Injectable Bone Cements

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INTRODUCTION: Injectable bone cements are indicated in vertebroplasty. However, as these materials have progressive setting, it is not clear how injectability can be maintained in service. The objectives of this research were: a) to monitor by ultrasound the setting of calcium sulfate cement; and b) to study the effect of mixing conditions on both the cement's setting and injectability.

METHODS: Cements were made by mixing calcium sulfate hemihydrate (CSH) with water at different L/P ratios ($0.5 \leq L/P(mL/g) \leq 3.5$). The setting was monitored by ultrasound during 1 h. Cement at L/P=2 mL/g was selected for further studies. The cement set under static conditions (i.e. without further mixing) to obtain the initial and final setting times (IST, FST). Then, replicas were prepared and set up to resting times RT<IST (RT<15 min). After completion of RTs, cements were mixed at 1600 rpm during 30 s and monitored again during 1 h. The evolution of the speed of sound was measured with this method. The injectability was obtained by extrusion using a testing machine at a crosshead speed of 50 mm/min up to maximum load of 300 N.

RESULTS: IST and FST increased with the L/P ratio. Speed of sound at saturation increased as the L/P ratio decreased, i.e. cements set faster and were more compact for lower L/P ratios.

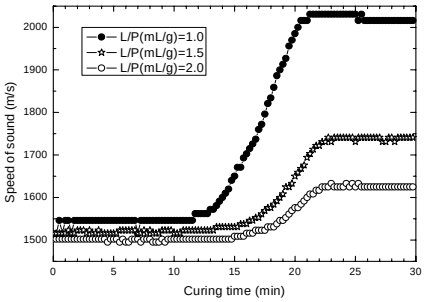

Fig. 1: Speed of sound versus the curing time: Effect of the L/P ratio for CS-cement.

Fig. 2 shows that setting times are drastically reduced if appropriate mixing protocols are applied to the initial mixture of the cement phases. Figs. 3 and 4 show that the injectability decreased with the increase of RT. Additional mixing (A) also significantly decreased the injectability as compared with the cement control with no additional mixing (N).

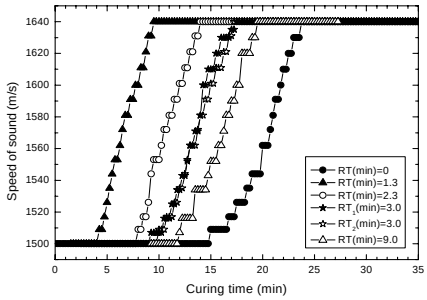

Fig. 2: Speed of sound versus the curing time: Effect of RT for CS-cement at L/P=2 mL/g.

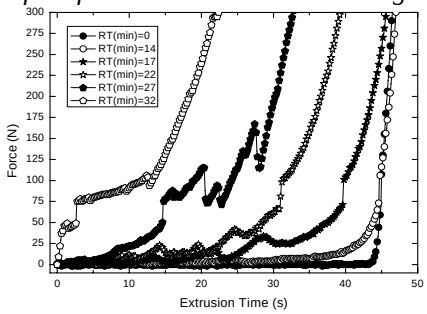

Fig. 3: Force versus the extrusion time: Effect of RT on CS-cement injectability at L/P=2 mL/g.

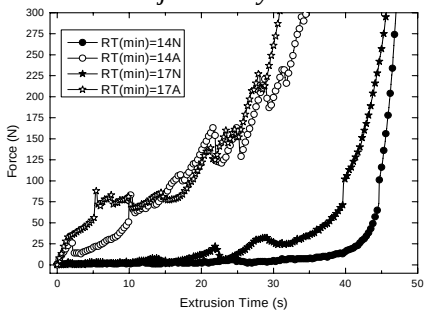

Fig. 4: Force versus the extrusion time: Effect of mixing conditions on CS-cement injectability.

DISCUSSION & CONCLUSIONS: It has been put forward that further mixing after cement constituency drastically affects the initial setting properties and the injectability of calcium sulfate (CS) cements. Ultrasound monitoring was able to follow the setting evolution accurately. The method was better than the Gillmore needles standard used to measure the IST and FST. The injectability of CS-cements was improved by continuous mixing of the cement's phases.

Acknowlegments

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Nanostructured multilayer nitride coatings for biocompatible materials

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INTRODUCTION: Most transition metals form binary or ternary nitrides, with good mechanical, tribological, anticorrosive and biocompatibility properties [1,2]. Therefore, nitrides coatings are well suited to the protection of the medical implant surfaces. The aim of this work is to improve the characteristics of a Ti alloy (Ti5Al2.5Fe) by depositing nanometer - scale multilayered TiN/TiAlN and Ti/TiN/TiAlN hard films (with individual layer thicknesses of about 8 nm).

METHODS: The multilayer thin films were deposited using magnetron sputtering technique using Ti and Ti+Al cathode targets [3]. The multilayers were obtained by using two adequate shutters, periodically opened and closed. The overall thickness of all coatings was approx. 3 μm. Chemical composition of the films was investigated by AES (Auger electron spectroscopy). Phase composition and texture were determined by X-ray diffraction. Microhardness measurements were performed using a microhardness tester at 20 g load. Scratch tests under standard conditions were undertaken to determine the coating adhesion. The wear behavior was investigated by using a testing apparatus consisting of a coated sample pressed on a rotating steel disc.

The flow cytometry technique was used in order to determinate the biocompatibility by measuring the cell viability in Calcein-AM test. The cytotoxic effects were determined by cellular models *in vitro* (MTT test).

RESULTS: The AES analysis showed that the multilayers are formed by almost stoichiometric layers (N/Ti≈N/(Ti+Al) ≈1). In the TiAlN films, the Al/Ti ratio was of about 0.8. The both types of multilayers exhibited a strong (111) preferred orientation. In the case of Ti/TiN/TiAlN coatings, Ti peaks were also detected. Microhardness $HV_{0.02}$ values were in the range 18÷21 GPa - TiN, 24÷26 GPa - TiAlN, 25÷27.2 GPa - TiN/TiAlN and 28÷34 GPa - Ti/TiN/TiAlN. A good adhesion of all films was found, critical loads of 46 – 55 N being measured. The best wear resistance and the lowest friction coefficient were measured for the Ti/TiN/TiAlN multilayer (0.18÷0.26) followed by TiN/TiAlN (0.22÷0.28), TiN (0.26÷0.30) and TiAlN (0.28÷0.34). The biological tests had shown a relatively high cytotoxic effect of Ti5Al2.5Fe alloy (cell viability 49%). The best cell viability was found for the Ti/TiN/TiAlN coatings (75%), followed by TiN/TiAlN (68%), TiN (65%) and TiAlN (63%).

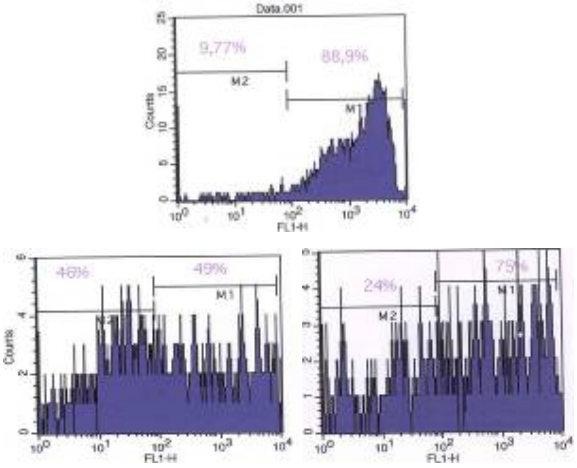

Fig. 1: Dermal fibroblasts: suspension (up), in presence of Ti5Al2.5Fe (left) and in presence of Ti/TiN/TiAlN (right).

DISCUSSION & CONCLUSIONS:
All the investigated coatings decreased the cytotoxic effects of Ti5Al2.5Fe alloy, while enhancing its mechanical, tribological properties. As compared with single layer coatings (TiN and TiAlN), the both multilayer films exhibited a superior mechanical, tribological and biocompatibility properties. Ti/TiN/TiAlN nanostructured multilayer presented the best biocompatibility properties and the higher wear resistance.

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