

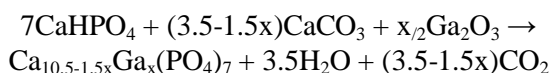
Gallium-doped calcium phosphates as a new approach for the local delivery of antiresorptive species

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INTRODUCTION: Calcium phosphate-based materials with suitable composition and porosity are currently used extensively as bioactive implants in human bone surgery, since they are slowly resorbed in the body and replaced by natural bone. If combined with drugs, such materials when implanted might in addition be also well-suited to address bone-related diseases. Given our recent studies showing that Ga³⁺ ions in solution exhibited a dose-dependent anti-resorptive effect [1, 2], we have investigated combination of gallium to calcium phosphates, which could offer a potential route to induce local Ga³⁺-mediated bone resorption inhibition in osteoporotic sites.

METHODS: Preparation of gallium-doped β -TCP ceramics: In a mortar, anhydrous calcium phosphate (0.174 mol) was intimately mixed with calcium carbonate and gallium oxide so that the (Ca+Ga)/P molar ratio corresponds to the desired x value according to eq. 1 ($x < 0.7$).



The mixture was ground for 30 min. and sintered at 1000°C for 24 hours (heating and cooling rates : 5°C / min).

RESULTS: Many studies have shown that gallium inhibits bone resorption, and gallium nitrate is currently marketed by Genta for the treatment of clearly symptomatic cancer-related hypercalcemia. While gallium nitrate acts as a potent inhibitor of bone density breakdown, it has however a very low bioavailability and thus requires a long and continuous IV administration. Our purpose was to design an implantable gallium-doped scaffold usable for the release of gallium *in vivo* to offer a better bioavailability of Ga(III) ions, in osteoporotic sites. Indeed, addition of a gallium precursor in the reaction protocols used for the preparation of β -tricalcium phosphate (β -TCP) or calcium deficient apatite (CDA) led to the novel gallium-doped analogues [3]. Suitable protocols were also developed to combine gallium ions to

injectable calcium phosphate cements (CPCs), showing a short cohesion time and setting times appropriate for pre-clinical uses [4]. No adverse effect occurred after implantation of this Ga-doped CPC in a small animal model (Fig. 1).

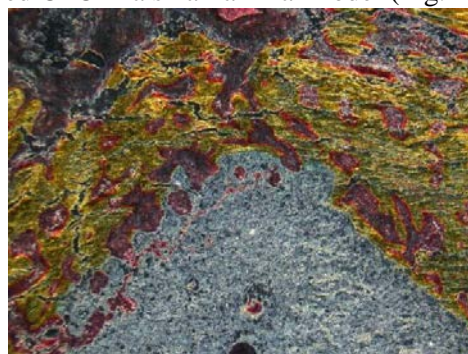


Fig. 1: Osteocoalescent interface between a Ga-doped CPC (in grey) and newly formed bone (yellow green) – undecalcified histology, Movat's staining. Substitution of the ceramic by active newly formed bone is observed in rabbit distal femur (4 weeks implantation)

DISCUSSION & CONCLUSIONS: Novel routes were designed for introducing gallium ions in various calcium phosphates, including TCP ceramics and injectable CPCs. In the latter case, taking into account the stability range of Ga³⁺ ions in solution, they interfere with the hardening reaction, by trapping phosphate ions released during cement setting. Original approaches were thus developed to incorporate gallium in the cement composition, without affecting the setting reaction. Preliminary *in vivo* investigation of the cement properties showed the ability of the gallium-loaded cement to resorb while promoting new bone formation.

REFERENCES: ¹ Verron et al., *British J. Pharm.* **2010**, *159*, 1681. ² Verron et al., *Biochem Pharmacol.* **2011**, in press. ³ Mellier et al. *Inorg. Chem.* **2011**, *50*, 8252. ⁴ Patents WO2010100209 and WO2010100211.

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Injectable hyaluronic acid hydrogel for bone tissue engineering

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INTRODUCTION: Injectable gels composed of extracellular matrix (ECM) have attracted much interest in the field of regenerative medicine [1]. We have previously focused our efforts to develop ECM mimetic hydrogel using hydrazone chemistry and have demonstrated its application for bone tissue engineering. These hydrazone crosslinked hyaluronic acid (HA) hydrogel has shown to be an efficient carrier of bone morphogenetic protein-2 (BMP-2) and formed bone in vivo within 6 weeks when injected below the rat periosteum [2]. One of the problems with such material design was the excessive swelling of the material, which also results in fast degradation of the scaffold, limiting the potential of the biomaterial for efficient tissue regeneration. Thus designing stable biomaterial scaffold with least chemical modifications of the ECM component is extremely important for regenerative medicine.

METHODS: Synthesis of hydrazide derivatives of hyaluronic acid

Hyaluronic acid (408 mg, 1 mmol of disaccharide repeating units) was dissolved in 100 ml de-ionized water at room temperature. Dihydrazide (1 mmol, carbonyldihydrazide or CDH/adipicdihydrazide or ADH) was added followed by HOBt (153 mg, 1 mmol). Thereafter, pH of the resultant solution was adjusted to 4.7 and solid EDC (19.17 mg, 0.1 mmol) was added and stirred overnight. The solution was loaded into a dialysis bag (Spectra Por-6, MWCO 3500) and dialyzed against dilute HCl (pH=3.5) containing 0.1 M NaCl (2×2L, 48 h), then dialyzed against deionized water (2×2L, 24 h). The solution was lyophilized and 360-390 mg HA-hydrazide derivative (6-8 % modification) was obtained.

A modified procedure was adopted for HA-oxalyldihydrazide or ODH as only 2.6 % coupling was obtained under the same reaction condition. In order to secure 7-8% modifications we optimized the reaction condition. Reaction was performed at pH 4.0 with double amount of EDC (38.3mg, 0.2 mmol), while other conditions remained same. HA-ODH was obtained in 8.2% modification.

RESULTS: Hydrogels were developed by mixing equal amounts of HA hydrazide derivatives and previously synthesized HA aldehyde derivatives [2]. Among these gels, HA-CDH gel was found to be most stable which did not swell significantly even in cell culture medium having 10% foetal bovine serum. This gel also showed superior degradation behaviour in presence of hyaluronidase, the enzyme known to degrade HA. This material was also shown to stabilize bone morphogenetic protein-2 (BMP-2) in vivo and form ectopic bone subcutaneously with oriented collagen.

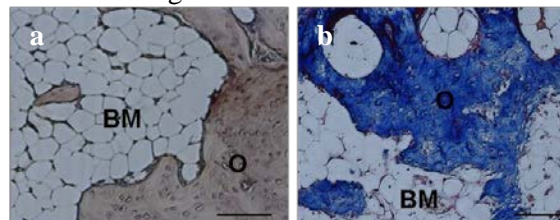


Fig. 1: Ectopic bone harvested after 7 weeks post implantation. The representative cross-sections were stained with (a) hematoxylin/eosin and (b) Masson's trichome

DISCUSSION & CONCLUSIONS: New type of hydrazone chemistry has been devised to develop HA-hydrogel with superior swelling and degradation properties than conventional hydrazone crosslinked gel. The improved performance of this material was also evident from the in vivo experiments in rat subcutaneous model for BMP-2 delivery. The uniqueness of this chemistry is due to the fact that, it stabilizes the N2 positive charge of hydrazone linkage by extended delocalization of the charge. This type of chemical linkage strategy using commercially available material could be envisaged for linking other biomolecules under mild physiological conditions.

REFERENCES: ¹ ES Place et al., Nature Mat. 2009, 8:457-70. ²E. Martínez-Sanz et al., 2011, 152:232-40

Antibiotic-loaded calcium phosphate cements and foams: Relationship between porosity and release behaviour

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INTRODUCTION: Calcium phosphate cements (CPCs) are used as synthetic bone grafts, due to their intrinsic osteoconductive properties [1]. The possibility of using CPCs as carriers for local delivery of antibiotics to the musculoskeletal system is attractive as prophylactic strategy or as treatment against existing infections [2-3]. Introduction of macropores by foaming CPCs may improve fluid circulation within the CPC, possibly leading to better performance as drug carriers and advantages for potential cell colonization.

It is the aim of this work to synthesize and characterize antibiotic-loaded CPCs (both dense and macroporous), as well as evaluating the performance of both systems as drug eluting materials.

METHODS: α -TCP was prepared as detailed elsewhere [4]. The liquid phase, MilliQ water, was added and manually mixed to obtain dense CPCs (C55). Macroporous CPCs (FC55) were prepared by addition of a surfactant (Tween80, Sigma Aldrich) to the liquid phase, mechanically foaming the latter and thus mixing with the powder phase. A constant liquid to powder ratio was fixed at 0.55mL/g. For antibiotic loaded CPCs (coded as A), Doxycycline hyclate (Sigma-Aldrich) was added to the α -TCP powder.

Total porosity (TotalP) and macroporosity (macroP) were determined by density measurements whereas the percentage of interconnections (IC) area was measured by image analysis using Matlab®. Dissolution test was adapted for drug release studies, which were carried out in a paddle dissolution tester (Pharma Alliance group, USA) following the European Pharmacopea. Quantification of doxycycline was done through spectrophotometric analysis at $\lambda=351$ nm.

RESULTS: Both dense and foamed antibiotic loaded CPCs were produced. Release results of CPCs loaded with 50mg of doxycycline per mL of liquid phase are presented (Fig. 1). Porosity, Macroporosity and Interconnection percentage (in area) results are presented in table 1.

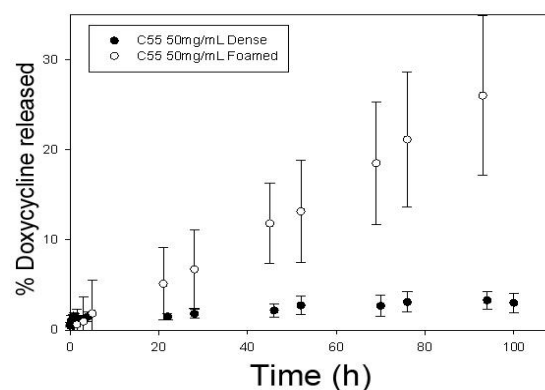


Fig. 1: Release of doxycycline from dense and foamed CPCs.

Table 1: Porosity features in dense (C) and foamed (FC) CPCs and with doxycycline (A)

	TotalP (%)	macroP (%)	IC (%)
C55	55.94 \pm 0.37	-	-
AC55	56.45 \pm 0.19	-	-
FC55	73.88 \pm 4.38	40.73 \pm 9.94	2.87 \pm 0.52
AFC55	62.93 \pm 4.09	14.40 \pm 9.43	12.27 \pm 2.60

DISCUSSION & CONCLUSIONS: Synthesis of both dense and macroporous CPCs with antibiotics is possible, and in both cases microporosity is present. Foamed CPCs release a higher percentage of doxycycline than dense CPCs. This can be explained by the macroporosity and interconnectivity introduced by foaming which may enhance fluid circulation within the CPC.

REFERENCES: ¹Ginebra et al., Acta Biomater. 2010, 6: 2863-73. ²Verron et al., Drug Discov. Today 2010, 15 547-552. ³Ginebra et al., J Control. Release 2006 113 102-110. ⁴Espanol et al., Acta Biomater. 2009, 5: 2752-2762.

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Injectable, Settable Polymer/ β -TCP Bone Grafts for Delivery of rhBMP-2

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INTRODUCTION: Recombinant human bone morphogenetic growth factor-2 (rhBMP-2) stimulates osteoblast differentiation and new bone formation. Biodegradable polyurethane (PUR) scaffolds carrying rhBMP-2 have been reported to support new bone growth when implanted in segmental femoral defects in rats [1]. To create an injectable PUR bone graft, β -tricalcium phosphate (β -TCP), a resorbable, osteoconductive ceramic [2], was added to limit the expansion of the graft *in vivo* and enhance new bone formation. In the present study, we investigated the ability of an injectable PUR/ β -TCP composite carrying rhBMP-2 to heal 8-mm critical-size calvarial bone defects in rats.

METHODS: The biodegradable PUR was synthesized from a lysine triisocyanate (LTI)-PEG prepolymer, a polyester triol (900 g/mol), and triethylene diamine catalyst [1]. The prepolymer, polyester, and β -TCP (45 wt%, 150 μ m) were mixed for 60 s. The resulting paste was mixed with lyophilized rhBMP-2 powder and catalyst for another 60 s and injected into 8-mm critical-size calvarial defects in rats. Pores (characterized by SEM) were generated by the reaction of water with the prepolymer, resulting in the formation of gaseous CO₂. Animals were sacrificed at 4 weeks and new bone formation evaluated by radiographs, μ CT, and histology. Treatment groups included the PUR/TCP graft with and without 200 μ g/mL rhBMP-2.

RESULTS: The porosity of bone grafts is important for control of rhBMP-2 release and cellular infiltration. The porosity of PUR/TCP grafts injected *in vivo* was 44 \pm 5%, compared to 35 \pm 10% for materials cured *in vitro*, suggesting that the materials undergo controlled and predictable expansion. Sustained release of rhBMP-2 was achieved for 30 days *in vitro*. Representative μ CT images taken at 4 weeks (Fig. 1) show that the injected grafts completely filled the defect for samples with and without rhBMP-2 (n=13). In the PUR/TCP grafts, there is evidence of remodeling near the perimeter of the graft in contact with host bone, as suggested by the increased density (white color) near the host bone interface. Addition of rhBMP-2

resulted in 44% more new bone formation compared to the PUR/TCP graft, as well as bridging of the defect with new bone at 4 weeks (Fig. 1B). Consistent with the μ CT data, histological sections (Fig. 2) reveal bridging of the upper and lower surfaces of the PUR/TCP+rhBMP-2 grafts with new bone.

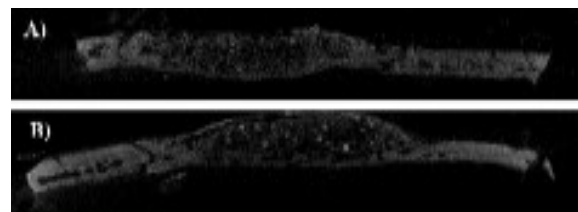


Fig. 1: μ CT images at 4 weeks. A) PUR/TCP, B) PUR/TCP+rhBMP-2.

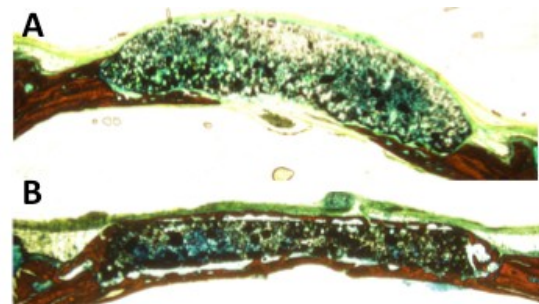


Fig. 2: Histological sections of A) PUR/TCP and B) PUR/TCP+rhBMP-2 grafts at 4 weeks stained with Sanderson's rapid bond stain.

DISCUSSION & CONCLUSIONS: Injectable PUR/TCP grafts support new bone formation and remodeling in critical-size rat calvarial defects. Addition of rhBMP-2 to the graft enhances new bone formation, which underscores the potential of these materials as an injectable, settable carrier for rhBMP-2. In ongoing experiments we are investigating the effects of rhBMP-2 dose on new bone formation.

REFERENCES: ¹ Li B. Biomaterials, 2009, 30: 6768-79. ² Bashoor-Zedah M. Biomaterials. 2011, 32(27): 6362-73.

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Functionalized calcium phosphates: the role of crystal structure.

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INTRODUCTION: Thanks to their excellent biocompatibility and bioactivity, calcium orthophosphates are widely employed in the preparation of biomaterials for hard tissues substitution and repair. Improvement of tissue response can be achieved through the development of functionalized calcium phosphate materials.

METHODS: Hydroxyapatite (HA) and octacalcium phosphate (OCP) were prepared by direct synthesis in aqueous solutions [1]. Functionalized calcium phosphates were synthesized through suitable modifications of the standard procedures [2-4]. Powder X-ray diffraction, TEM, spectrophotometric, BET, thermal and chemical analyses were used to characterize the structure, morphology and composition of the products.

RESULTS: *Ionic substitutions.* Strontium is quantitatively incorporated into HA in the whole range of composition, where its isomorphous substitution for calcium provokes a linear increase in the lattice parameters, which is coherent with the greater ionic radius. On the contrary, OCP structure allows for a partial substitution of Sr for Ca up to 7.4 at%, because of the presence of lattice water.

Acidic macromolecules. Acidic polyelectrolytes can be considered synthetic analogs of acidic biomacromolecules. Polyacrylic acid and polyaspartic acid (PASP) display only a minor inhibitory effect towards the growth of HA crystals from aqueous solution, and are incorporated into the inorganic crystals through a specific interaction. The results of the structural and morphological analyses indicate a greater reduction of the dimensions of the coherently scattering domains and of the crystals sizes along the direction perpendicular to the *c*-axis (Fig. 1). On the contrary, the presence of PASP strongly inhibits the synthesis of OCP in aqueous solution, and provokes remarkable morphological and structural modifications.

Bisphosphonates. Bisphosphonates not only prevent bone dissolution, but might play a role

on the deposition of the bone mineral phase as well. The direct synthesis of HA in their presence may be quite difficult due to their great affinity for calcium ions. As a matter of fact, the synthesis of hydroxyapatite in the presence of different bisphosphonates yields different products due to different structural interaction.

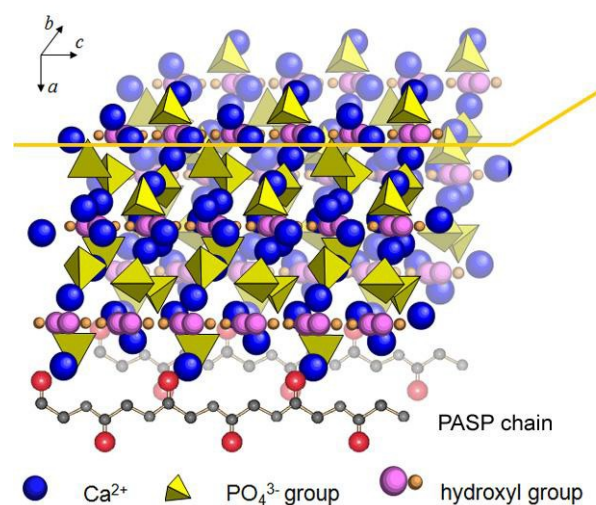


Fig. 1: Hypothesis of structural interaction between hydroxyapatite lattice and polyaspartate polymeric chain.

DISCUSSION & CONCLUSIONS: the various structures of calcium phosphates account for the quite different incorporation of ions, as well as molecules, into the final materials. Similarly, the structures of two potent bisphosphonates, alendronate and zoledronate, justify their different interaction with HA nanocrystals.

REFERENCES: ¹ E. Boanini, M. Gazzano, A. Bigi (2010) *Acta Biomater* **6**:1882-94. ² A. Bigi, E. Boanini, M. Gazzano, M.A. Kojdecki, K. Rubini (2004) *J Mater Chem* **14**:274-9. ³ E. Boanini, M. Gazzano, K. Rubini, A. Bigi (2007) *Adv Mater* **19**:2499 - 2502. ⁴ E. Boanini, P. Torricelli, M. Gazzano, M. Fini, A. Bigi (2012) *Biomaterials* **33**:722-30.

Degradation & characterisation of new biphasic calcium phosphate cements

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INTRODUCTION: Some biphasic calcium phosphate ceramics (BCP), composed of hydroxyapatite (HA) and β -tricalcium phosphate (β -TCP), have been shown to be osteoinductive [1]. This means that they are able to induce bone formation by stimulating differentiation of stem cells towards the osteoblastic lineage. Both composition and microstructure play a relevant role in this behaviour, through the modulation of mechanisms such as Ca^{2+} release [2], and protein retention, which are key factors in stem cell differentiation. The aim of this work is to develop new biphasic calcium phosphate cements (BCPCs) with adequate composition and textural properties, which could join the benefits of osteoinductive ceramics with the advantages associated to calcium phosphate cements. To evaluate the influence of composition on Ca^{2+} release, accelerated degradation was performed.

METHODS: α -TCP and β -TCP were mechanically mixed in different α -TCP/ β -TCP ratios of: 20/80, 40/60, 80/20; 0/100 and 100/0 were used as controls. BCPCs were obtained by mixing powder and an aqueous solution (2% w/v of Na_2HPO_4) at a liquid/powder ratio (L/P) of 0.35. Subsequently, the BCPCs were placed in cylindrical moulds and immersed in Ringer's solution for 7 days. Physical, chemical and mechanical properties were characterised. Accelerated degradation studies were performed at pH=2 in order to simulate osteoclastic bone resorption [3]. Weight loss and Ca^{2+} release were evaluated.

RESULTS: XRD revealed that, after setting, BCPC consisted of mixtures of HA and β -TCP. Thus, α -TCP completely hydrolysed to HA, whereas β -TCP remained unreacted. Specific surface area of BCPCs decreased with increasing β -TCP content (*Fig 1*). The final microstructure was strongly affected by β -TCP percentage, evolving from plate-like to needle-like microstructure, the β -TCP particles remaining hidden within the HA matrix.

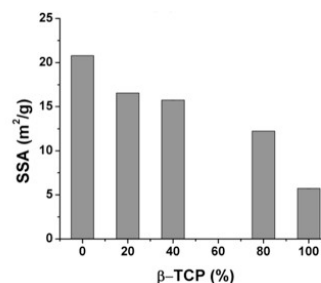


Fig. 1: Specific surface area of the set BCPCs.

Accelerated degradation of BCPCs showed a significantly higher weight loss in pure β -TCP (*Figure 2*). No significant differences in calcium ion concentration were detected among BCPCs.

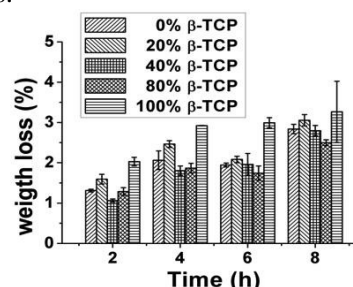


Fig. 2: Effect of composition changes on BCPCs dissolution in acid medium (pH=2).

DISCUSSION & CONCLUSIONS: The introduction of β -TCP affected the final microstructure and the physical and chemical properties of BCPCs. β -TCP particles remained “hidden” within the material, as they may act as nucleation points for calcium deficient HA. Although pure β -TCP lost more weight with time, no differences were observed for other BCPC. This was attributed both the lower SSA of BCPC, and to the fact that the more soluble β -TCP was “hidden” within the structure.

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Study on silicate bioceramics

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INTRODUCTION: The silicon containing bioactive glasses and glass ceramics have drawn more attention in the recent years due to their excellent bioactivity and the ability to induce the formation of hydroxycarbonate apatite on the surface of the materials, which bone can bond when implanted into the body. More recently, studies have shown that the dissolution products of these bioactive materials stimulated bone cell proliferation, differentiation, and gene expression related to tissue regeneration, and this stimulatory effect may be considered as a new criterion for evaluation of bioactivity. However, some problems such as unsuitable mechanical properties, low bioactivity and degradability still remain which limit the clinical application of these materials, in particular for the application in tissue engineering and in situ tissue regeneration. Therefore, development of new bioceramics with improved properties is of significant importance.

METHODS: Silicate bioceramic powders were prepared by chemical precipitation and sol-gel process. Ceramic discs were prepared by conventional sintering in air. MTT assay was performed to evaluate cell proliferation. The osteogenic effect of the silicate ceramics was evaluated by culturing bone marrow-derived mesenchymal stromal cells (BMSC) in presence of ceramics, and the osteogenic gene expression was analysed. In addition, in vivo evaluation was conducted by implantation of porous ceramics in a rabbit bone defect model and new bone formation was evaluated.

RESULTS:

MTT assay showed that silicate ceramic extract promoted proliferation of hBMSC significantly more than did β -TCP extract. The results of alkaline phosphatase (ALP) activity test and the expression of osteogenic marker genes demonstrated that the osteogenic differentiation of hBMSC was enhanced more by silicate ceramic extract than by β -TCP extract. In vivo, a

histomorphology analysis and histomorphometry of the porous bioceramics implants in a rabbit femur defect models indicated that both in early- and late- stage implantations, akermanite promoted more osteogenesis and biodegradation than did β -TCP; and in late-stage implantations, the rate of new bone formation was faster in silicate ceramics than in β -TCP.

DISCUSSION & CONCLUSIONS: These results indicated that silicate bioceramics stimulated osteogenic differentiation of BMSC, and suggest that these silicate bioceramics might be potential candidates for bone regeneration and tissue engineering applications.

A New way of Forming Calcium Phosphate Cements Using Bioactive Glasses as Reactive Precursors Part 1 Theory and Background Studies

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INTRODUCTION: Bioactive glasses have been investigated and used as bone substitute materials for over 40 years [1]. The capacity of bioactive glasses to stimulate bone formation is probably better than any other synthetic material; however, their clinical success has been hindered by two major problems i) they generate a high local pH and ii) the lack of any in situ setting property. By contrast calcium phosphate cements, CPC, have the ability to be shaped, moulded and even injected during surgical procedures. CPCs, however, lack sufficient strength and are often have slow setting characteristics. This paper will critically review the current views on the structure of bioactive glasses and the mechanisms of degradation and speed of apatite formation. The influence of network connectivity, phosphate sodium and fluoride content of the glass will be discussed in relation to the potential use of bioactive glasses as reactive precursors in calcium phosphate cement formation. These studies will then be used as the basis for the design of bioactive glasses for forming in situ setting cements in Part 2.

Glasses offer considerable advantages over calcium phosphate salts for the formation of cements. Most notably the amorphous nature of glasses results in increased reactivity and dissolution rate. The stoichiometry can be varied at will and many components that can not be readily obtained in soluble salt form can be incorporated into a soluble glass.

METHODS: The glasses for these studies were all produced by a melt quench route [2]. The glasses were ground to <38 microns and their ability to form apatite like phases in Tris buffer was investigated. The influence of Network Connectivity (NC) phosphate content sodium content and fluoride content were all investigated. The structure of the glasses was characterised by ³¹P and ¹⁹F solid state MAS-NMR and the ability to form apatite like phases was followed by XRD, FTIR and solid state NMR.

RESULTS: The MAS-NMR showed that phosphorus was always present as orthophosphate and the fluorine was present as F-M(n) species. XRD, FTIR and ³¹P MAS-NMR showed apatite like phases to be formed on immersion in Tris buffer with all the glasses including the sodium free glasses. The results show that the time to form an apatite-like phase increases with increasing NC. The time to form apatite-like phase decreased monotonically with increasing phosphate content. Incorporating fluoride in the glass appears to accelerate the formation of apatite and results in the formation of fluorapatite. ³¹P NMR spectra of selected glasses as a function of time enabled the kinetics of apatite formation to be followed by deconvolution of the NMR spectra. Fluorapatite was formed at times <3Hrs from fluoride and high phosphate glasses

DISCUSSION & CONCLUSIONS: Increasing the phosphate content and maintaining a low NC, incorporating fluoride and reducing the sodium content results in glasses that form apatite like phases much more rapidly than existing bioactive glasses and open the possibility of them being used to form a new generation of CPC cements.

Sodium is not required in the glass composition which contradicts the widely accepted mechanism proposed by Hench.

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Ultrasonication for the delivery of the hydraulic calcium-phosphate paste

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INTRODUCTION: The poor injectability of the calcium-phosphate paste is an important limitation for the use as bone graft substitutes [1,2]. Ultrasonication was applied to the paste through the plunger of the delivery syringe to improve the injectability. The study was run in large factorial design of experiments with the following factors: Factor A: LPR (38, 39, 40%); Factor B: Ultrasonication amplitude (0, 20, 25 and 30%); Factor C: Syringe (5 or 10mL). Three responses measured are: the injectability, injection force and power applied to paste. This abstract is to report on the results of this study.

METHODS: The injectability tests were performed using an 858 Mini Bionix II system (MTS Systems Corporation, Eden Prairie, MN) described in an earlier study [1]. Special fixtures were designed to hold the ultrasonic probe perpendicular to the syringe plunger. The sonotrode, also referred to as probe, of 6-mm diameter, and 113-mm length was geometrically made fit to syringe plungers. The syringe plungers are made of Delrin material and are used for the 5 and the 10-mL syringes. The 100-percent amplitude of the probe corresponded to a distance of 150 micrometers. The sonotrode was connected to ultrasonic processor (Cole-Parmer, USA) to control the ultrasonic amplitude. The ultrasonic processor produces up to 130 kW power at a frequency of 20 kHz. The statistical analysis of the factorial designs of experiments was performed using a software developed by Jacques Lemaître (EPFL, Switzerland; <http://ltp.epfl.ch/page-35623-en.html>). Results were considered significant for $p < 0.01$.

RESULTS: Small improvements were observed with the increase in the LPR and decrease in syringe size, which is consistent with the previously published results [1]. Improvements due to ultrasonication were significant and remarkable [Fig.1]. For the 5-mL syringe, the 30-percent ultrasonication amplitude increased the injected volume fraction to 99.6 ± 0.7 % for the 40-percent LPR

paste. For the 10-mL syringe, ultrasonication higher than the 20-percent amplitude led to an increase in the injected volume fraction up to 94.9 ± 0.7 % for the 40-percent LPR paste.

The statistical analysis of the results obtained at 20, 25 and 30% amplitude revealed that the injection force was significantly reduced and were more stable. Ultrasonication had also an effect on the LPR of the extrudate out of which samples were taken in 30-second intervals. Specifically, ultrasonication reduced the excess water in the extrudate and allowed for the delivery of a homogeneous product.

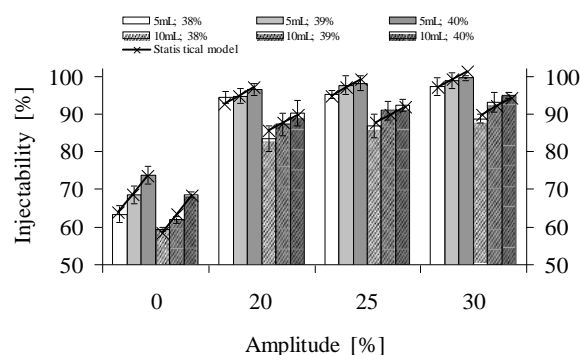


Fig.1: Injectability as a function of amplitude, LPR and syringe size. (x) Estimated values according to the statistical analysis of the factorial designs.

DISCUSSION & CONCLUSIONS: This study in a broader context of earlier studies [1,2], the enhancement of injectability seems to be related to the reduction in the injection force versus the force of the filtration process. Ultrasonication is believed to reduce the local compaction of the powder in the initial paste and thus leads to a more uniform paste in the syringe, which also enhances the extrudate homogeneity. Ultrasonication is an effective and practical solution to improve the delivery of the thick hydraulic paste in that it reduces the segregation of the two phases and allows for a more homogenous extrudate.

REFERENCES: ¹Habib M et al. Acta Biomater. (2008);4:1465-1471. ²Bohner M, Baroud G. Biomaterials (2005);26:1553-63

Self hardening Macroporous Biphasic Calcium Phosphate bone void filler for bone reconstruction. Animal study and Human data

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INTRODUCTION: Due to the lack of macroporosity in current available Calcium Phosphate cement used in osteoarticular surgery, Micro and Macroporous Biphasic CaP Cement (MCPC) was developed. The concept was the association of a settable fast resorbable matrix and a sieved fraction of microporous biphasic calcium phosphate (BCP) granules, recognized for osteogenic properties. During the matrix resorption a porous structure is created and the osteoconductive effect of the granules promotes bone ingrowth. The aim of this study was to test the MCPC in an animal model and to realize specific cases in vertebral body filling [1].

METHODS: A goat preclinical study was realized to evaluate the efficacy of MCPC concept for C3 and C4 vertebral body filling defects during 6 months. Light microscopy, MicroCT, SEM and histomorphometry were realized.

After ethic committee approval, five human clinical cases were performed. They all had a vertebral compression fracture according to AO classification without neuro disturbances. They were all operated in supine position using a minimal invasive technique: percutaneous insertion of 4 cannulated screws and vertebral body reduction using the balloon kyphoplasty technique. The void created by the balloon after reduction was filled by MCPC. X rays were performed, at immediate post op, then 3, 6 and 12 months of follow up. Bone healing and loss of correction were analyzed using the EOS low dose radiation system allowing 3D reconstruction and CT scan analysis.

RESULTS: In the goat vertebral model, bone remodelling was evidenced demonstrating the MCPC absorption and the osteogenicity at the expense of the cement and surrounding residual BCP granules. Bone trabeculae were observed coming from the spongy bone to the implant site.

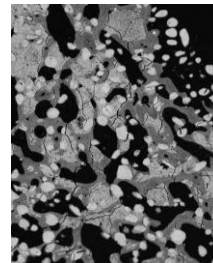


Fig. 1: MCPC in Goat C3 vertebral filling at 6 months

The human study demonstrated the biocompatibility and the safety of MCPC concept for bone reconstruction. Osteosynthesis was associated to prevent high mechanical constraints during the adsorption and bone ingrowth. More cases are needed to improve the mechanical construct to avoid the use of the brace.

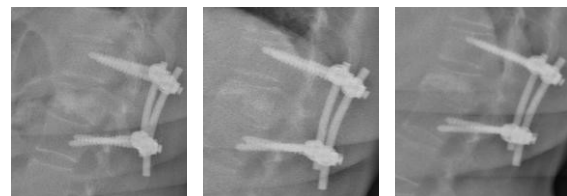


Fig. 2: Human vertebral filling, post op, 4 and 6 months X rays follow up

CONCLUSIONS: Results of this study demonstrated the importance of special combination of calcium phosphate granules in a self setting calcium phosphate bone void filler to provide macroporosity and scaffolding for newly formed bone.

REFERENCES: ¹ Daculsi G, Khairoun I, leGeros RZ, et al. Bone Ingrowth at The Expense of a Novel Macroporous Calcium Phosphate Cement. *Key Engineering Material*. 2007;330-332:811-4

New Palliative Intervention for Painful Metastatic Bone Disease The OsteoCool™ System

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INTRODUCTION: Spinal metastases can cause intractable skeletal and neuropathic pain. Some tumors exhibit limited or no response to existing therapies due to specific morphology or location. Thus, there is a clinical need for a minimally invasive treatment alternative in the management of spinal metastases.

Radiofrequency ablation (RFA) is a percutaneous technique that utilizes high frequency electrical current emitted from a probe, causing ionic tissue heating and subsequent cell death. RFA has been used to coagulate soft tissue tumors such as liver, lung, and kidney neoplasms. However, it is challenging to apply RFA in the spine since bone is less thermally and electrically conductive than soft tissue.

A new internally-cooled bipolar RF probe design allows the creation of large lesions in heterogeneous bone tumor tissues in a consistent and controlled manner.

METHODS: Efficacy of the bipolar RF ablation system was evaluated in a VX-2 metastatic rabbit carcinoma model.

To evaluate pre-clinical safety of the RFA system, treatment was then performed in a scaled-up healthy porcine model with vertebral dimensions relevant for human applications. First human cases were done under either general or local anesthesia. If mechanical stabilization was required, vertebral augmentation was performed subsequently.

RESULTS: All animal model procedures produced a controlled temperature response.

MRI analysis of treated rabbit femurs showed uniform ellipsoid lesions of 3x2 cm; histology revealed corresponding tumor cell death.

In the porcine vertebrae, peripheral tissues remained at body temperature throughout all treatments, showing that the thermal effect was isolated to the intended treatment area in the vertebral cortex. Post-treatment MRI revealed clinically and anatomically relevant lesions spanning half the vertebrae. All animals demonstrated normal behaviour during

neurological assessment following treatment, indicating sensitive neural structures surrounding the treatment area remained intact. Human cases were technically and clinically successful. The device placement was straightforward and performance of the Baylis RF Generator was as intended (*Fig 1.*). Patients tolerated the procedure well under local anesthesia and sedation.

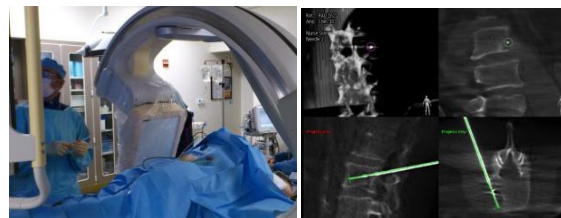


Fig. 1, Device placement was straightforward in human clinical cases

DISCUSSION & CONCLUSIONS: The OsteoCool system is the first clinically available RFA system that uses a single internally-cooled bipolar probe with temperature control for palliation of pain due to metastatic vertebral malignancies. RFA of vertebral metastases has been shown as an effective second-line treatment¹. This minimally invasive, quick and reliable method is advantageous in achieving symptom control in these medically and anatomically complicated patients.

The Baylis OsteoCool RF Ablation System has received regulatory clearance in the U.S., E.U. and Canada.

This novel, internally-cooled, bipolar probe design has proven to be effective at creating lesions in metastatic vertebral malignancies that are anatomically relevant in size and shape.

REFERENCES: ¹ Dupuy DE et al. Cancer, 2010, 116(4).

Pathogenesis and treatment of periprosthetic joint infection

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INTRODUCTION: Implant-associated infection is caused by surface-adhering bacteria persisting as biofilm. Implants are not rejected by the body. However, the host reacts with these foreign bodies, a process which is designated as biocompatibility. The interaction of the device with adjacent granulocytes and complement induces local inflammation and impairs local microbial clearance.

PATHOGENESIS: Despite laminar airflow technique and antibiotic prophylaxis during surgery, the infection rate is still as high as 0.5% after hip replacement and even 4-8% after ankle arthroplasty. This is due to the fact that 100 CFU *S. aureus* are enough to cause a permanent device-associated infection [1]. The presence of a foreign device increases the risk for infection at least 10⁵-fold [1]. This high susceptibility of implants to bacteria or fungi is due to a locally compromised host defence. The interaction of the non-phagocytosable implant with local granulocytes results in an impaired ingestion rate, impaired bactericidal activity, decreased superoxide production, and partial degranulation of these cells [1, 2]. Similarly, the polyethylene wear debris induce a local granulocyte defect, characterized by impaired uptake of *S. aureus* and a decreased bactericidal activity [cited in 2]. More recently, it has been shown that biofilm-embedded *S. epidermidis* were more resistant to the killing of normal neutrophils than the isogenic biofilm-negative *ica* mutant [cited in 2]. Due to the local immunodeficiency, prosthetic joints are life-long endangered by hematogenous seeding during sepsis. Indeed, the risk for hematogenous seeding on prosthetic joints is 34-39% during *S. aureus* bacteremia [cited in 2].

TREATMENT: The treatment goal is complete eradication of infection, freedom of pain, and correct function of the joint. Reaching this goal requires rapid diagnosis and a rational treatment strategy including adequate surgery (debridement, one-stage or two-stage exchange) combined with prolonged antibiotic therapy [3]. Surface adhering biofilms are highly resistant to host defence and antimicrobial agents [3, 4]. According to animal experiments and clinical studies, rifampin is more efficacious against surface adhering staphylococci

than other agents [3]. The risk for emergence of rifampin resistance should be minimized by correct use of this drug. Following a rational treatment algorithm, results in a 80-90% cure-rate of periprosthetic infection [3].

OUTLOOK: Given the limited efficacy of traditional antibiotics in implant-associated infections, novel strategies such as coating of the device, vaccination against biofilms, and quorum-sensing inhibitors are promising future options for prevention and treatment [4].

REFERENCES: ¹W. Zimmerli et al (1982) Pathogenesis of foreign body infection: description and characteristics of an animal model. *J Infect Dis* **146**:487-97. ²W. Zimmerli and P. Sendi (2011) Pathogenesis of implant-associated infection: the role of the host. *Semin Immunopathol* **33**:295-306. ³W. Zimmerli et al (2004) Prosthetic-joint infections. *N Engl J Med* **351**:1645-54. ⁴W. Zimmerli, and C. Moser (2012) Pathogenesis and treatment concepts of orthopaedic biofilm infections. *FEMS Immunol Med Microbiol* (Epub ahead of print).

Combination Percutaneous Threaded Screw and Methyl Methacrylate Stabilization of Cancer and Post Radiation Induced Pelvic Fractures

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INTRODUCTION: Treating cancer patients who have pain and disability related to pelvic insufficiency or pathologic fractures can be challenging. These fractures are often the result of either extensive prior pelvic radiation or lytic metastatic lesions. Surgical options carry significant morbidity in these patients, and can result in prolonged hospitalization. Hardware failure due to poor bone quality is a significant complication. Here we will present our experience with stabilization of pelvic fractures via image-guided percutaneous screw fixation and subsequent instillation of Polymethyl methacrylate (PMMA).

METHODS: Seven consecutive patients presented with insufficiency or pathologic pelvic fractures. These patients were seen in either the orthopedic oncology clinic or interventional radiology/ oncology clinic for percutaneous pelvic stabilization. Using a combination of CT and CT-fluoroscopic guidance, an interventional radiologist and orthopedic surgeon acting as co-surgeons percutaneously threaded stabilization screws across the pelvic fractures followed by PMMA injection post placement to aid in hardware stabilization.

RESULTS: All seven patients had successful percutaneous placement of 4-7 mm stabilization screws for fracture fixation. In contrast to open surgical fixation, we had no need for general anesthesia, no significant blood loss (< 50 mL), and no cardiovascular complications seen frequently in orthopedic surgical procedures. In follow-up, all patients have reported marked improvement in pain scores, decreased narcotic usage, and increased mobility. All patients were eligible based on their intervention for discharge home the day of the procedure; however, some required short hospitalizations range of 2-4 days due to other scheduled procedures. At the time of this report, no hardware failures have occurred.

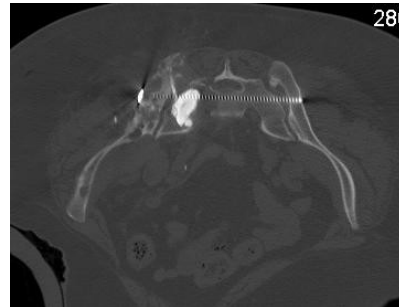


Fig. 1: CT-fluoroscopically guided percutaneous transsacral threaded screw and PMMA stabilization in renal cancer

DISCUSSION & CONCLUSIONS:

Sacroplasty and osteoplasty have been shown to relieve pain¹; however, the structural integrity of the underlying bone after cement augmentation is not significantly improved. Similarly, fluoroscopic surgical pelvic screw fixation rarely incorporates PMMA installation due to fluoroscopic anatomic limitations and concern for cement leakage. Advanced image guidance allows the opportunity to combine these complimentary methods. Therefore, we believe that this less invasive percutaneous hybrid screw and cement augmentation affords more structural support allowing for better long term results.

REFERENCES:

¹Percutaneous sacroplasty
Amar C Gupta, Albert J Yoo, Jeffrey Stone, John C Barr, Allan Brook, Sean Tutton, Orlando Ortiz, Ariel E Hirsch, Mykol Larvie, Michael E Frey, Mahesh V Jayaraman, Joshua A Hirsch

*J Neurointervent Surg*2011; published at <http://jn.is.bmj.com> on October 15, 2011 (10.1136/neurintsurg-2011-010136).

ACKNOWLEDGEMENTS: is effort would not be feasible without the close collaborative relationship between the Division of Vascular and Interventional Radiology and Department of Orthopaedic Surgery at the Medical College of Wisconsin.

Vertebral augmentation with targeted cement deposition technique after channels creation using a nitinol wire, the “Blazer” system

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INTRODUCTION: Both vertebroplasty and balloon kyphoplasty have been described for the treatment of vertebral compression fractures. Vertebroplasty is known for its high leakage rate compared with balloon kyphoplasty. Patients with severe osteoporosis treated with kyphoplasty had showed a potential for re-fracture due to stress shielding effect [1], beside the reported limited value of height restoration

In this study we are reporting our early experience with a new innovative vertebral augmentation system that preserves cancellous bone architecture by using a percutaneously introduced nitinol wire designed to create channels within the vertebral body. The channels created in the bone are intended to direct the flow of PMMA bone cement in a targeted fashion inside the vertebral body.

METHODS: Retrospective multicenter analysis of 41 patients, (23 women and 18 men, average age of 73 years), with a total of 48 levels were included in the study. 35 patients had benign osteoporotic compression fractures and 6 patients had malignant lesions. 6 patients had very sclerotic bones, usually due to osteoblastic metastasis and 9 patients had intra-vertebral clefts. All patients were treated using the “Blazer” vertebral augmentation system (Benvenue Medical, Santa Clare, CA, USA). All patients but one were treated using unipedicular access. The robust nitinol wire is used to create channels and arcs inside the cancellous bone, which can cross the midline to the contra-lateral side or bridge through bone clefts inside the vertebral bodies. Cement deposition intended to follow the created channels to allow targeted delivery. Visual Analog Scores (VAS) was obtained before and within 2-4 weeks after the procedure.

RESULTS: The Blazer system allows full control with wide safety margin for the nitinol wire to create strategic channels to enhance targeted cement delivery and enables bone cement to interdigitate into the cancellous bone

and stabilizes the fracture. The system was found to be particularly useful in creating channels to connect clefts with normal adjacent bone as well as creating channels in hard sclerotic bones as seen in blastic metastasis. No significant leakage was identified. Patients had statistically significant reduction of VAS after the procedure.

DISCUSSION AND CONCLUSION: The Blazer system is an innovative approach for treatment of compression fractures. It allows a unipedicular access with creation of channels that results in a uniform distribution and interdigitation of the cement in the cancellous bone. This type of cement augmentation allows more control over the cement delivery. The nitinol wires are safe to use and well controlled from outside the body. The Blazer system is particularly helpful in augmentation of sclerotic bone due to the sharp stiff nature of the nitinol wire and it's ability to penetrate easily through hard bone as well as in the treatment of cases with intra-vertebral clefts where the created channels can bridge the cleft with the normal surrounding bone to avoid cleft enlargement around the cement bolus or its extrusion.

REFERENCES: ¹Becker S, et al., J Miner Stoff Wech 2011; 18(suppl.):9-12

Comparison between radiofrequency targeted vertebral augmentation, balloon kyphoplasty and vertebroplasty using high viscosity cement in treatment of vertebral compression fractures

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INTRODUCTION: Both vertebroplasty and balloon kyphoplasty have been described for treatment of vertebral compression fractures. Targeting and control of cement delivery has long been a topic of debate and goal of minimally invasive treatment of such fractures. Balloon kyphoplasty was developed to create a cavity within cement can be contained. Vertebroplasty is known for its high leakage rate compared with balloon kyphoplasty. In vitro studies have shown that high-viscosity cements significantly decrease the incidence of leakage in cancellous bonelike substrates compared with low-viscosity cements [1]. Also creation of channels inside the cancellous bone may help reduce the effect of stress shielding in osteoporotic fractures [2]. In this study we compare the incidence and pattern of cement leakage in cases treated with high-viscosity cement vertebroplasty (HVC-VP), standard balloon kyphoplasty (BKP) and a novel targeted vertebral augmentation procedure, Radiofrequency Targeted Vertebral Augmentation (RF-TVA) using the StabiliT Vertebral Augmentation System, DFINE, San Jose, CA.

METHODS: Retrospective evaluation of postoperative radiographs of patients treated with the three techniques was analyzed for the incidence and location of cement leakage. 112 consecutive patients with 159 treated levels were included in this review. There were 66 HVC-VP levels, 46 levels in BKP, and 47 levels in RF-TVA groups treated, ranged from T3 to L5.

RESULTS: In the HVC-VP group, a total of 33 leakages were reported (17 discal, 11 venous, 4 paravertebral and 1 epidural). In the BKP group a total of 31 leakages were reported (15 discal, 11 venous, 3 paravertebral and 2 epidural). In the RF-TVA group, a total of 16 leakages were reported, (8 discal, 5 venous, 3 paravertebral and non epidural). No significant leakage that required any surgical intervention was noted.

DISCUSSION AND CONCLUSIONS:

These findings show that targeted cement augmentation using RF-TVA technique using the StabiliT system may provide approximately 50% reduction in leakage rate when compared to conventional VP using high viscosity cement and standard BKP. These results may be related to the unique combination of controlled delivery of radiofrequency activated (very high viscosity) cement at a fixed low rate of injection into site-specific channels created using a navigational osteotome. RF-TVA allows uni-pedicular access and remotely controlled cement delivery to decrease procedural invasiveness and physician radiation exposure, respectively

ABBREVIATIONS: HVC-VP; high viscosity cement vertebroplasty, BKP; balloon kyphoplasty, RF-TVA; radiofrequency targeted vertebral augmentation.

REFERENCES: ¹Baroud G, et al., Spine 2006; 31:2562– 68. ²Becker S, et al., J Miner Stoff Wech 2011; 18(suppl.):9-12

Early experience with a new intravertebral PEEK / biomaterial implant

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INTRODUCTION: Balloon kyphoplasty (BKP) has revolutionised the treatment of osteoporotic vertebral fractures over the last decade. However one of the major criticism of the technique was always the additional destruction of intact vertebral bone [1]. Vertebroplasty uses PMMA cement without additional bone destruction and has shown to have the same clinical outcome as BKP. We report the clinical outcome of an advanced kyphoplasty system, which enables height restoration and stabilisation without additional bone destruction [2].

This system allows the use of all different resorbable or non – resorbable bone cements and may also act as a drug – delivery platform.

METHODS: Prospective single arm study. 23 subsequent patients with 36 vertebral fractures (3 metastasis, 20 patients with osteoporotic fractures) were treated prospectively with 39 kivaplasties (Benvenue Inc., Santa Clara, USA). 13 single, 7 double (3 prophylactic), two triple and one quadruple level were performed on 22 lumbar, and 17 thoracic levels. Mean patient age 71.6 years (41 – 87 y, male 68,7, female 74y, 10 male and 13 female patients). Follow up radiologically and clinically (SF36, ODI, VAS) after 6 weeks and 12 months.

RESULTS: 1 subsidence and one adjacent and one remote fracture occurred within the follow-up period. The SF 36 scores showed no statistically improvement after 6 weeks ($p=0.17$), but after 12 months ($p=0.01$), whereas the ODI scores were significant after six weeks and 12 months ($p=0.01$) as well as the VAS scores, which dropped from pre-op 6.2 to 3.3 up to one year ($p=0.02$). An average of 1.7ml PMMA was injected.

No intra- or postoperative complications were noted.

DISCUSSION & CONCLUSIONS: Kivaplasty represents an advanced kyphoplasty techniques with the additional benefit of avoiding additional bone damage. Furthermore it allows minimisation of the cement volume which is reducing local and systemic cement toxicity effect. We used also resorbable bone

cements outside of this study with similar clinical outcome. Clinical outcomes are comparable to other kypho- or vertebroplasty techniques [3].

REFERENCES: ¹ Dabirrahmani et al. Mechanical Variables Affecting Balloon Kyphoplasty Outcome- A Finite Element Study. *Comput Methods Biomech Biomed Engin.* 2011 Apr 1:1. ² Wilson et al. An ex vivo biomechanical comparison of a novel vertebral compression fracture treatment system to kyphoplasty. ³Rosales Olivares et al. Vertebral augmentation treatment of painful osteoporotic compression fractures with the Kiva VCF Treatment System. *SAS Journal* 2011 5,4: 114-119

ACKNOWLEDGEMENTS: This study was financed by a grant from Benvenue Medical.

Screw Augmentation using CaP Injectable Bone Cement

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INTRODUCTION: While many papers present empirical data for orthopaedic screw pullout from bone, our aim is to understand the fundamental phenomena that determine the wide variation of pull-out force that exists *in vitro* and *in vivo*. This paper presents mechanisms for the augmentation of cancellous bone and screws or anchors inserted into it. Pull-out tests of screws from a range of configurations of augmented cancellous bone substitute are described. Finite element models representative of these configurations are presented, along with associated parametric variation.

METHODS: The experiments were carried out on a range of Sawbones® bone substitute materials, with and without a simulated cortical layer. HydroSet® calcium phosphate bone cement was used to fill pre-drilled holes, and 4mm bone screws inserted. In one series of the tests, ballistic gelatine was used to represent marrow, and hence restrict the radial flow of cement within the Sawbones polymer. Where layered materials were used simulate a cortical shell, these were machined to produce reduced skin stiffness.

Finite element models of cement-augmented cancellous bone with and without cortical layers have been developed using ANSYS commercially available software. The models use both real bone geometry (developed from CT scans and subsequent processing) and idealised simplified models. These enable ready variation of key parameters that determine screw pull-out. Degree of radial augmentation and placement of cement are examined.

RESULTS: The test results confirm [1,2] the importance of the cortical layer and the positioning of any bone augmentation placed next to it or near it.

Simplified finite element models [3] show the importance of the radial penetration of the cement, while the presence of gelatine (representing fluids in the pore space) restricts this radial flow of injected cement.



Fig. 1: A 4mm screw in cancellous bone substitute augmented with HydroSet® showing the displaced dome of cement.

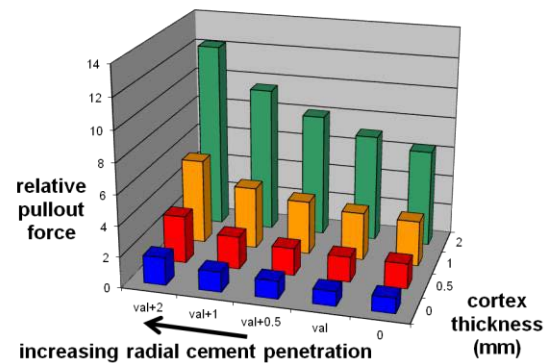


Fig. 2: Radial penetration affects pull-out force.

DISCUSSION & CONCLUSIONS: One key result is that the cortical layer contributes significantly to holding power of screw which may be of clinical significance. Even if screws are not engaged with this layer, the stiffening effect of the underlying cancellous bone is critical to strength.

REFERENCES: ¹Seebeck et al., Osteoporos Int (2005) (DOI 10.1007/s00198-004-1777-0) ²Stadelman et al. J Biomech. doi: 10.1016/j.jbiomech.2010.07.025. ³Brown et al., Comp Meth in Biomech and Biomedical Eng. DOI: 10.1080/10255842.2011.624516

ACKNOWLEDGEMENTS: Some work presented has been funded by studentships from the Stryker Corporation.

Injectable and flexible tricalcium phosphate/biopolymer composites for bone regeneration

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INTRODUCTION: The injectability of calcium phosphate cements (CPCs) has been widely studied for minimal invasive applications [1]. However, injectability is also a relevant parameter in rapid prototyping applications. The strategy adopted in order to improve CPC's injectability is to incorporate a soluble biopolymer in the liquid phase increasing its viscosity and stabilizing the calcium phosphate particles. Thus, the aim of the work was to develop and optimize CPC formulations to produce self-setting robocasted scaffolds for bone tissue engineering.

METHODS: Alpha tricalcium phosphate (α -TCP) was obtained by solid-state reaction and used as solid phase. Either water or 2.5wt% Na_2HPO_4 aqueous solution together with 10 or 20wt% B-type gelatine solutions were employed as liquid phases. Injectability test was performed as described elsewhere using a syringe with a 2 mm aperture [2]. The extrusion was started 2.5 minutes after mixing the liquid with the powder phase ($L/P = 0.6$ and $0.8 \text{ mL} \cdot \text{g}^{-1}$). Injectabilities and injection forces were determined. After optimization of the formulation, three dimensional (3D) scaffolds were robocasted using a rapid prototyping machine (3Dn Series, NScript, USA) with tapered tips of 0.84 mm of inner diameter (i.d.; Nordson EFD). The paste was dispensed controlling the displacement of the pump at 10 mm min^{-1} .

RESULTS: The incorporation of gelatine in the liquid phase increased considerably the injectability of the CPC and increased the injection force (table 1), allowing injection through thin cannulas up to 0.84 mm. The amount of gelatine (10 vs 20wt%) did not influence significantly the injectability or injection forces but increased the cohesion of the injected strand, as did Na_2HPO_4 . As shown in Fig 1, flexible strands were obtained right after injection and before α -TCP setting. The strand diameter was easily controlled by

varying the cannula's inner diameter, allowing the fabrication of 3D random or organised scaffolds, with controlled porosity and interconnectivity by robocasting.

Table 1. Differences of injectability between CPC with and without gelatine.

Mat: α -TCP L/P=0.6	% Inj.i.d.	Inj Force
	2mm	(N)
0% gel	73 ± 2.0	7 ± 1
10wt% gel	93 ± 1.5	21 ± 6
20wt% gel	93 ± 2	20 ± 3
0% gel + Na_2HPO_4	65 ± 2.0	7 ± 1
10wt% gel+ Na_2HPO_4	$92,5 \pm 1,8$	25 ± 9
20wt% gel+ Na_2HPO_4	94 ± 2	32 ± 6

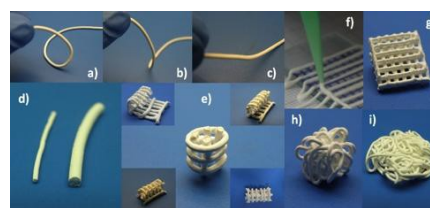


Fig. 1: Extruded flexible strands with different diameters a-d, Folded 3D structures e, Scaffold with controlled porosity f-g, Random coil scaffolds h-i.

DISCUSSION & CONCLUSIONS: α -TCP was employed together with gelatine to produce fully injectable calcium phosphate composites with self-setting ability that exhibit flexible features right after injection. Their injectability is considerably superior to CPCs without gelatine. This is relevant not only for minimally invasive grafting procedures, but also for robocasting applications, to produce scaffolds with controlled or random porosities and interconnectivities for tissue engineering.

REFERENCES: ¹Bohner M. and Baroud G., *Biomaterials*, 2005; 26:1553-1563. ²Khairoun I. *et al.*, *JMSMM*.1998;9:425-428.

ACKNOWLEDGEMENTS: Authors acknowledge the MICINN for the financial support through MAT 2009-13547 project.

Vertebroplasty: Dependency of the Subsequent Fracture Risk Reduction on the Injection Location

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¹ [Institute for Biomechanics, ETH Zurich, Zurich, CH,](#)

INTRODUCTION: Vertebral fragility fractures are often treated by injecting bone cement, typically polymethylmethacrylate (PMMA), into the collapsed vertebral bodies (vertebroplasty). Subsequent fractures of the augmented, adjacent or remote levels immediately following the augmentation have been reported and altered load patterns are considered as one of the main causes [1]. To which extent cement type, cement volume, cannula placement and other augmentation parameters affect these load patterns, is, to a large extent, unknown. The aim of this study was twofold. First, to investigate and introduce methods that allow pre-computing realistic cement distributions with a high accuracy, even in a clinical setting with only limited computational power available. Second, to explore and find, in combination with an adequate mechanical FE model of the bone-cement composite [2] and a corresponding failure criterion, treatment parameters and the consequent cement spreading pattern that minimize the occurrence of subsequent fractures within the cemented or adjacent vertebral levels.

METHODS: The flow of a non-Newtonian fluid in trabecular bone is governed by a modified version of Darcy's law. This formulation implies several flow parameters to be calculated, e.g. the bone permeability. [3], fluid interface positions [4] and non-linear rheology properties. We generally approach this issue by mapping pre-computed micro-scale values to the macroscopic length scale. The mechanical response of the bone was determined by applying adequate load boundary conditions and material properties derived from local bone morphometry and a rule of mixture law to a specimen-specific FE model. The risk of fracture is defined as the ratio of the actual stress value divided by the absolute bone strength and has been calculated for every element in the finite element model.

RESULTS: The cement distribution patterns related to two different injection sites are shown in Fig. 1. The location of the first ce-

ment cloud has been predicted more laterally within the vertebral body; hence loads acting on the endplates are more non-uniformly transferred across the vertebra, compared to the second cement distribution.

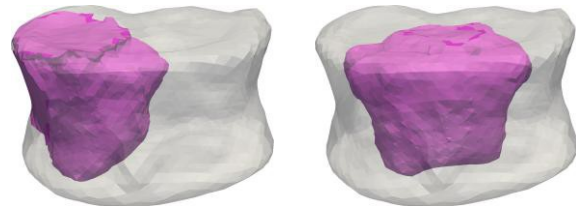


Fig. 1: Cement distributions for two different injection locations within a L1 vertebral body.

The risk of fracture distribution prior to and after augmentation within the vertebral body is indicated in Fig. 2. A relevant reduction of the subsequent fracture risk is only achieved with the second augmentation scenario.

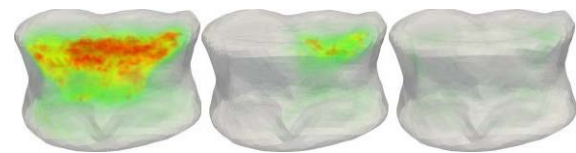


Fig. 2: Risk of fracture determined prior (left) and after augmentation (middle, right)

DISCUSSION & CONCLUSIONS: With this study, we demonstrate that the magnitude of fracture risk reduction and consequently the effectiveness of the vertebroplasty procedure are sensitive to the injection site. A mistakenly selected injection position has the potential to provoke a subsequent fracture at the treated or adjacent spine levels. Until now, this aspect has not been given enough attention in clinics, science and recent debates on the efficacy of vertebroplasty.

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Development of a new method for imaging the bone-cement interface under load for FE model validation

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INTRODUCTION: Previous studies [1, 2] have demonstrated that continuum-level finite element models of whole vertebral bodies are capable of predicting strength and stiffness with a good degree of accuracy. However, when the same approach is used to generate models of vertebrae following augmentation with cement, the models consistently over-estimate the stiffness and strength [2]. The aim of this study was to develop a method of imaging cement-bone specimens under load using micro-computed tomography (μ CT) to gain a greater understanding of the micromechanical processes at the cement-bone interface and enable more representative continuum-level finite element models to be developed.

METHODS: A custom screw-driven compression device was developed to enable bone or synthetic specimens to be incrementally deformed under displacement control. The device was fabricated from a mixture of Delrin, Perspex and stainless steel, with the central region containing the specimen fabricated entirely from Delrin and Perspex to minimise x-ray back scatter under μ CT scanning and avoid image artefacts. The device incorporated a thrust bearing to ensure that no torques were applied to the specimen, and a load cell to record the forces required to produce a given deformation. The device was developed following a proof-of-concept study conducted with a simpler compression device, used to image specimens comprising open-cell polyurethane foam (Sawbones AB, Sweden) augmented with PMMA bone cement. Cylindrical specimens (13mm diameter, 20mm height) were cut from Sawbone and potted in Delrin end caps to minimise end-effects [3]. PMMA was injected into each specimen until a roughly spherical bolus was formed. Each specimen was then incrementally loaded in the compression device. After each increment, the entire device was imaged using μ CT. In this way it was possible to build up a series of 3-dimensional images of each specimen which allowed characterisation of the cement-bone

interfacial deformation and strain distribution as the load was increased.

RESULTS: Typical images of an augmented Sawbone specimen are shown in Fig 1. In this case, it was observed that the majority of the deformation occurred in the synthetic bone above the cement bolus.

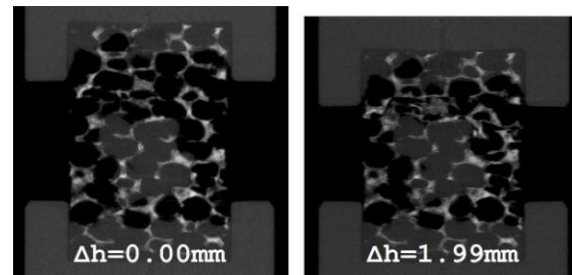


Fig. 1: Composite images created by combining a number of μ CT cross-sections through a Sawbone specimen augmented with PMMA bone cement: Un-deformed specimen (left), 1.99 mm applied displacement (right).

DISCUSSION & CONCLUSIONS: The results of the proof-of-concept study demonstrated that it was possible to incrementally deform and image cement-augmented specimens to investigate the mechanical properties of the bone/cement interface. The greatly reduced stiffness of Sawbone when compared to actual trabecular bone and the likely significant differences at the post-augmentation interface due to in-vivo conditions necessitated the re-design of the compression device. Further study of specimens of Ovine lumbar vertebral trabecular bone fabricated and augmented in the same manner as in the proof-of-concept-study are now underway.

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ACKNOWLEDGEMENTS: This study was funded through an EPSRC studentship.

A validated finite element study comparing the use of calcium phosphate and PMMA cements for the augmentation of traumatically fractured vertebrae.

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INTRODUCTION: This study combines a computational and experimental approach to assess the stiffness and strength of fractured porcine vertebrae following vertebroplasty using calcium phosphate (CaP) or PMMA cement. The stress distributions pre and post augmentation within the vertebrae are also evaluated.

METHODS: Three-vertebra porcine specimens (n = 18) were fractured, imaged and tested according to methods described in previous studies [1, 2]. Three cement types were investigated: two CaP formulations [3] (type A and type B, Modulus (E) = 1.01 and 0.585 GPa respectively) and a lab grade PMMA cement (type C, E = 1.035). Each cement type was injected into six fractured specimens. The specimens were then imaged again and tested under axial compression.

The image datasets collected for all 18 specimens both pre- and post-augmentation were converted into FE models. The material properties and volume of the cements were then manipulated for each model, to assess the factor that most affected stiffness of the specimens. In addition, the stress within the vertebral bodies was analysed and compared between specimens in the fractured, and various augmented cases to determine the effect the different cements have on the distribution and magnitude of the stress concentrations within the vertebrae. Stress distributions resulting from axial loading of intact specimens obtained in a previous study were then used to compare the effectiveness of the cement augmentation to an undamaged case.

RESULTS: From the experimental tests, the respective increases in stiffness and strength after augmentation compared to the post-fracture, pre-augmentation values were 1% and 9% for Type A, 13% and 43% for Type B and 51% and 42% for Type C cements.

The mean stress within the bone elements of the fractured vertebra was found to be similar to the intact vertebra case, in both the anterior/posterior regions and whole vertebrae; however, the variability in stress is larger in the fractured vertebrae, Fig 1.

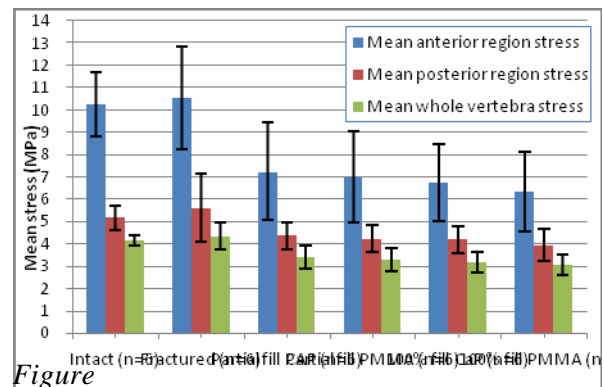


Figure 1. Mean stress – whole

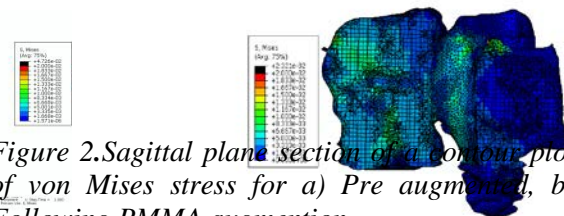


Figure 2. Sagittal plane section of a contour plot of von Mises stress for a) Pre augmented, b) Following PMMA augmentation

DISCUSSION & CONCLUSIONS:

Following augmentation the mean von Mises stress within the vertebra decreases, this is most likely due to a proportion of load being supported by the injected cement. This could raise the issue of stress shielding within the vertebral body. The results show high regions of von Mises stress within the bone, localised in the superior and inferior regions, proximal to the cement, Figures 2 and 3. Further investigation is required to determine how alterations in the stress distribution within the vertebra under loading, can effect bone remodelling in the long term.

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ACKNOWLEDGEMENTS: Funded by the EPSRC (UK).

Effect of Cement Augmentation technique on the Fatigue and Pullout Strength of Pedicle Screws

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INTRODUCTION: Spinal implants have a significantly higher failure rate than the widely successful hip and knee implants. Pedicle screw fixation is the most often used intra-operative stabilization technique of the posterior spine and loosening at the bone-screw interface is their most prevalent complication. Many studies testing the fixation of pedicle screws have only reported on the pullout strength; even though pedicle screw pullout is not a common clinical failure mode. The most efficient method to increase pullout strength is to augment the screw with cement; however, the relation of cement augmentation technique to fixation strength is still unclear. The aim of this study was to investigate how different augmentation techniques alter the fatigue and pullout strengths of pedicle screws.

METHODS: Osteoporotic single lumbar vertebrae (n=27, BMD= 91.61 ± 37.97gHA/cm³) were bi-laterally instrumented and augmented under fluoroscope guidance with pedicle screws (Ø=5.5mm, length=50mm, TangoRS™, Ulrich Medical, Ulm, Germany). Vertebrae were assigned to 3 groups to achieve comparable volumetric BMD and spine levels: A) no cement, B) vertebroplasty augmentation C) augmentation through the screw (Figure 1). Specimens first underwent sinusoidal fatigue loading at the screw head in the cranial and caudal directions (Bionix I, MTS, USA). Compressive, sinusoidal loading was increased step-wise starting with a range of 25N to 75N with an increase of the upper value by 25N every 250 cycles (1 Hz). The fatigue loading regime was based on *in vivo* loading ranges [1]. Fatigue testing was terminated when the pedicle screw rotated more than 20°. The contralateral side was subsequently loaded in pure axial pullout using displacement control (5mm/min) until a peak force was reached.

RESULTS: Volumetric BMD significantly affected pullout strength ($r^2=0.17$, $p=0.036$), fatigue load ($r^2=0.44$, $p<0.001$), and stiffness

($r^2=0.45$, $p<0.001$). Fatigue failure load correlated with the respective pullout strength ($r^2=0.29$, $p=0.004$). Both augmentation techniques exhibited a significant increase in pullout force from the no cement group (Figure 1). Augmentation through the screw yielded the best fatigue results; however, neither augmentation method significantly improved fatigue failure ($p>0.48$). Stiffness positively correlated with fatigue load ($r^2=0.37$, $p=0.001$).

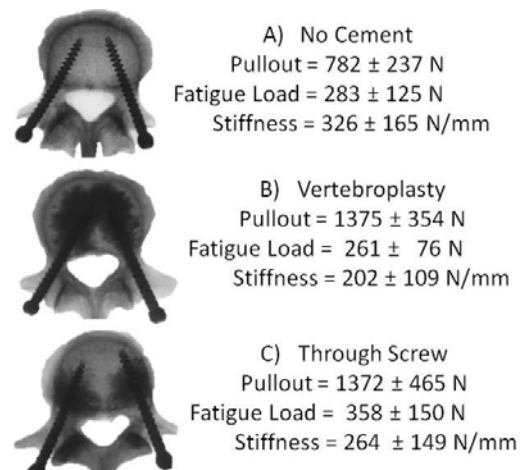


Fig. 1: Radiographic examples from each group with pullout, fatigue and stiffness results.

DISCUSSION & CONCLUSIONS: Both augmentation methods increased pullout strength by over 175%; however, large standard deviations prevented significance in fatigue testing. The distinctive cement distribution patterns exhibited by the various techniques (Fig. 1) might be responsible for the tentatively better results for the through screw augmentation group. An increased sample size would be necessary for trend confirmation. Increasing stiffness of the screw-bone construct could lead to an increase of fatigue strength.

REFERENCES: ¹ Rohlmann et al., Spine, 2000, 25:2981-2986.

ACKNOWLEDGEMENTS: EU funding from the Marie Curie Action- SpineFX (238690), screws from Ulrich Medical and cement from TECRES Medical are kindly acknowledged.

Vascular network formation in tissue engineered constructs using endothelial-like-cells derived from mesenchymal stromal cells

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INTRODUCTION: Human mesenchymal stromal cells (hMSCs) are adult cells that can be isolated from the bone marrow, expanded *ex vivo* and differentiated into various cell types. Therefore hMSCs are increasingly used in regenerative medicine as a cell source for restoring worn-out or damaged tissues such as cartilage, cardiac muscle or bone. We investigated the potential of hMSCs as a source for endothelial cells that can be used to create a vascular network within a graft, restore damaged vessel networks in ischemic limbs and improve vascularization of islets of Langerhans.

METHODS: Recently, we have developed a protocol for the differentiation of an immortalized line of MSCs (endothelial growth medium combined with Matrigel cultures) as demonstrated by induction of CD31, KDR and vWF expression, tube formation on Matrigel and AcLDL uptake. Cells that were differentiated according to this protocol are referred to as endothelial-like MSCs (EL-MSCs). Subsequently, we also screened hMSCs from 20 donors to investigate whether MSCs are responsive to this protocol and to determine inter-donor variation regarding the ability to differentiate towards endothelial progenitors.

RESULTS: Our results indicate that our protocol is reproducible in MSCs and can be used for differentiation with more than 90% positive outcomes. EL-MSCs obtained according to our protocol were tested in 4 different delivery systems to investigate whether they can be used in different applications. We performed 3 different *in vivo* experiments in nude mice. The chosen injectable ways of delivery were EL-MSCs in Matrigel, EL-MSCs on collagen modules (Fig.1) and EL-MSCs embedded in dextran gel. We also studied one method combining Matrigel and PLLA/PLGL scaffolds.

Additionally, we have tested *in vitro* if EL-MSCs can improve vascularization of islets of Langerhans. This is crucial for maintaining islets survival after implantation and to improve therapeutic outcome of diabetic patients concomitantly. Our results showed that coating islets with EL-MSCs improves their sprouting potential compared to previously used HUVECs/MSCs coatings. We are currently performing *in vivo* experiments to confirm our approach as promising in clinical applications.



Fig. 1: Vessels within collagen modules.

We are also looking into possible application of EL-MSCs in bone tissue engineering by examining whether they can improve *in vivo* bone formation.

DISCUSSION & CONCLUSIONS: To summarize, our results suggest that EL-MSCs are a promising candidate for obtaining endothelial-like cells that can be used for improving vascularization in various applications including potential use in therapies.

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Intraosseous forming implants for bone metastases treatment through locally combined hyperthermia and chemotherapy

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INTRODUCTION: Bone metastases might be efficiently treated using intraosseous implants. In this view, we propose novel formulations that, once injected intratumorally, form a solid implant. Poly(methylmethacrylate) cements and cellulose acetate organogels are two relevant formulations already used in vertebroplasty and embolization procedures, respectively. They can be loaded with both an anticancer agent (doxorubicin DOX) and superparamagnetic beads (SSB) for combining chemotherapy and hyperthermia, the latter being an effective adjuvant in cancer therapy [1].

METHODS: Cement was prepared by mixing poly(methylmethacrylate) and its monomer in the presence of an initiator and an activator. Organogels were prepared using cellulose acetate solution in DMSO (20% w/v). For both types of implants, SSB and DOX loadings were 40% (w/v) and 2.5% (w/w), respectively. *In vitro* drug release was carried out in a saline media (NaCl 0.9%) at 37 °C. *In vitro* toxicity of the implants was tested using the XTT proliferation kit. Immortalized human prostate cancer cells, PC3, were exposed to DOX for 24h before the cell viability was measured and compared with a control of non-treated cells.

RESULTS: PMMA cements and organogels were able to generate heat in the range of 41 to 42 °C and displayed sustained DOX release over 10 days (Fig. 1). The release profiles were not influenced by the heat generated during a 25 min-hyperthermia session at 6 mT and 140kHz, allowing further *in vitro* studies on the synergetic effects of hyperthermia and chemotherapy. The heating power of the implants, so-called specific power loss (SPL), indicates the potential for hyperthermia-induced antitumoral effect [2]. *In vitro* toxicity on PC3 cells showed preserved drug bioactivity, resulting in up to 40% and 75% cell death after 24h exposure to the elution medium for cement and organogels, respectively.

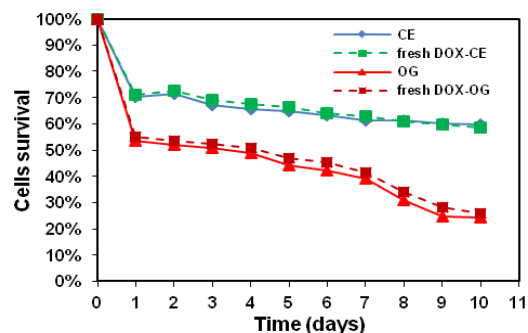


Fig. 1: Cell viability of PC3 cells after 24-hour incubation either in implant-released DOX (plain lines) from cement (CE) and organogels (OG), or in a fresh DOX solution (dashed lines) at equivalent concentration (n=3).

Cement for intraosseous injection might provide mechanical support to the weakened bone, in contrast to organogels, which would be more suitable for soft tissue tumors.

Table 1. Comparative properties of both formulations (n=3).

	Organogel	Cement
Elasticity [MPa]	0.128 ± 0.024	798 ± 10
SPL [W/g]	0.438 ± 0.024	0.519 ± 0.017

DISCUSSION & CONCLUSIONS: Two different approaches for *in situ* forming implants were evaluated. Two types of implants were successfully loaded with DOX and SSB, providing a sustained anticancer agent delivery and potentially cytotoxic temperatures. These data show within clinically acceptable conditions the feasibility of combining hyperthermia with local anticancer agent release.

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A New way of Forming Calcium Phosphate Cements Using Bioactive Glasses as Reactive Precursors: Part 2 Experimental Cements

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INTRODUCTION: The first successful calcium phosphate cement (CPC) was developed by Chow and Brown [1] and was based on a composition comprising of ground $\text{Ca}_4(\text{PO}_4)_2\text{O}$ and CaHPO_4 salts that formed apatite. This study investigates CPCs produced using melt derived bioactive glasses as one of the initial reagents. Using bioactive glasses as one of the starting materials has three main advantages. (i) Unlike a crystalline salt, the composition of a glass is not limited by stoichiometry and can be varied at will. (ii) Glasses are generally more reactive and dissolve more quickly than their crystalline counterparts. (iii) It is possible to incorporate many species into a glass; notable examples include strontium, zinc, cobalt and fluoride.

METHODS: Glasses were prepared as described previously [2]. Cements were prepared by mixing the glass(s) and $\text{Ca}(\text{H}_2\text{PO}_4)_2$. This powder was then mixed with 2.5% solution of Na_2HPO_4 solution to give a L/P of 0.8 and an overall molar ratio of Ca/P = 1.67. After the Na_2HPO_4 solution was pipetted into the powder mixture, the paste was mixed using a spatula on a glass slab for 30 seconds. Setting times and compressive strength was measured on cement samples after 1h, 1d, 7d, 28d immersion in TRIS buffer solution. X-ray diffraction and ^{31}P & ^{19}F MAS NMR was performed on each cement samples.

Table 1. Glass Compositions for Cements (mole %)

Glass	SiO_2	P_2O_5	CaO	Na_2O	CaF_2
NFRII	36.0	7.00	52.00	5.00	0.00
WFRII	33.5	7.00	49.72	4.78	5.00

RESULTS: X-ray diffraction and ^{31}P MAS NMR showed the cement produced using the fluorine free glass NFRII set to form octacalcium phosphate (OCP). Cement setting times were measured at 6 min for the initial setting and 18 min for the final setting times. Investigation of the influence of the overall Ca/P demonstrated that a Ca/P of 1.67 yielded the highest compressive strength. Alteration of

Ca/P influenced nature of the phases formed in the cements, e.g. brushite formed at the Ca/P <1.56.

The cement with the fluoride containing glass WFRII set to form fluorapatite. X-ray diffraction showed that an apatite had formed and that OCP was not present, ^{19}F MAS NMR demonstrated the apatite was fluorapatite, FAP. The setting time of this cement was measured as 8 min for initial set and 27 min for final set.

DISCUSSION & CONCLUSIONS: Fluoride is known to inhibit OCP formation and favour the formation of apatite. This is consistent with the fluorine free glass based cement forming OCP whilst the fluoride containing glass forms FAP. These initial results demonstrate the feasibility of producing CPCs using a bioactive glass as one of the reagents as an alternative to using calcium phosphate salts. The initial results show good setting times and compressive strengths, essential for biomedical applications. The formation of the OCP phase with the fluoride free glasses is favorable for bioactivity and bioresorption. In contrast the formation of FAP may be desirable where less resorbable and more durable cement is required for example as a restorative dental filling material as opposed to a bone substitute application.

The ability to incorporated therapeutic ions is an especially attractive proposition given past research incorporating fluoride and strontium ions. These new glass based CPCs offer considerable promise and scope for further development.

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CFD-Simulation of Percutaneous Vertebroplasty

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INTRODUCTION: Success in bone augmentation procedures depends much upon the experience of the physician and upon cement behaviour. A comprehensive numerical investigation of the injection and curing process can contribute to a better understanding of cement distribution and heat generation within the vertebral body. But above all, it can prevent potential risks, such as cement leakage and heat necrosis among others. The detailed insight into these procedures can guide the physician to effectively perform the intervention.

METHODS: Micro-CT scans of human vertebral bodies, affected by osteoporosis, were converted with aid of the image toolbox Amira to get digital geometric data. These triangulated surface files can be further processed by established meshing tools for simulation programs. Furthermore, a commercial bone cement was analysed with respect to its viscous behaviour, thermal expansion, chemical shrinkage and thermodynamic properties, e.g. specific heat capacity and thermal conductivity. A special focus was laid on capillary and rotational rheometry, to study the complex flow behaviour. Based on these experiments, material equations have been derived and adapted according to the measurements [1].

The open source CFD-toolbox OpenFOAM was chosen to simulate the injection process of bone cement into vertebral bodies, since it offers the user free accessibility to implement own material equations. In particular the volume of fluid solver (VoF) interFoam was extended by a heat transfer equation as well as by customized viscosity models for bone cements [2]. At the end of the injection process simulation the results, i.e. the cement and temperature distribution within the vertebral body are passed to a finite element simulation, which is capable to represent the curing process of the cement, including chemical shrinkage,

thermal expansion, exothermal heat generation and internal stresses.

RESULTS: For initial CFD-simulations an extract of $3 \times 3 \times 3 \text{ mm}^3$ was separated from the entire vertebra model, in order to save computing time. Calculation results show clearly the flow front between cement and bone marrow (Figure 1). The distribution of cement and temperature can be tracked during the whole injection process.

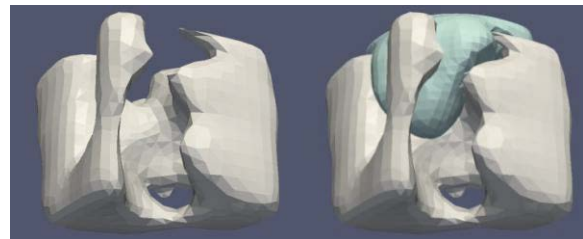


Fig. 1: $3 \times 3 \times 3 \text{ mm}^3$ cut-out model of cancellous bone structure before injection (left) and partly filled with bone cement (right)

DISCUSSION & CONCLUSIONS: True-to-detail simulation of the percutaneous vertebroplastic surgery process visualizes the physical processes going on in vivo during cement injection and curing. Moreover, it offers the physician to run through different virtual interventions varying the injection time, pressure, needle position etc. and thus find optimum process parameters.

The application of more complex geometries up to more than one vertebral body and the definition of accurate boundary conditions are demanding future work.

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ACKNOWLEDGEMENTS: This research is funded by the German Research Foundation (DFG) within the project PAK 273.

Enhancing screw fixation in poor quality cancellous bone: *Present and future options*

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INTRODUCTION: The concept of enhancing orthopaedic screw fixation using injectable cements is not new. For decades compelling preclinical evidence has been presented in the literature. Despite the potential benefit there has not been any significant clinical adoption of this technique. Current issues are that injectables are only qualified as non-load bearing void fillers and additionally they are not approved (in USA) for use with screws in poor quality bone.

THE CLINICAL ISSUE: The main clinical issue in orthopaedic procedures where screws are used is screw loosening/migration in poor quality bone. For example, in proximal humeral locking plates screw cut-out ranges from 15 to 40% [1] Orthopaedic bone screw fixation needs to be sufficient both early (days/weeks) and late (weeks/months), as well as requiring easy removal at a potential revision procedure. The author estimates that more than 100 million orthopaedic screws are implanted worldwide and these are increasingly at risk of migration and loosening in poor quality bone.

ENHANCED FIXATION STRATEGIES: The technique of injection of cement into a predrilled hole, prior to screw insertion, has been developed using different materials such as polymers and calcium phosphate cements. For example, Cortoss was shown to be effective in augmenting stripped screws in fracture plating of the osteopenic ankle [2]. In addition, PMMA has been suggested to enable earlier weight bearing in osteopenic ankle fractures [3]. The high failure rate of locked plates in the proximal humerus (>40% in the >60 age group) was unexpected³ as these implants were believed to be effective in osteopenic bone. A recent retrospective review by Egol et al [4] suggests that calcium phosphate cement may be very effective in reducing the risk of locked plate migration in subgroup of proximal humeral fractures. The reluctance of surgeons to use injectable cement for augmenting cancellous bone may be partly due to lack of appropriate regulatory approval for this indication. This concern lead the author to run a project to establish the first European approval for calcium phosphate cement (HydroSet, Stryker) intended to augment orthopaedic screws in cancellous bone, both at and after surgery. This

claim was established in Europe in 2008 however at the time of writing there is no such approval for screw augmentation with any class of injectable cement in the United States.

DISCUSSION: The opportunity for injectable cement augmentation of screws in poor quality bone appears to be obvious. However the lack of regulatory approvals and corresponding clinical evidence has slowed the adoption of this technique. The proximal humerus and the ankle are both good indications with clinical evidence to support them. Proximal femoral fractures may also be a good target but clinical evidence is lacking at present. In the future one can imagine injectable cement/drug combinations. PMMA and antibiotic combinations already exist. Additionally Graftys (France) plan to do a human clinical evaluation combining calcium phosphate cement together with a bisphosphonate. Moreover it may be anticipated that the recent alliance of J&J, Synthes and Eli Lilly (2011) will eventually establish bone augmentation through pharmaceutical means in combination devices that elute bone changing drugs. These companies have, between them, the competences and resources necessary to establish such combination medical devices. This would be a paradigm shift in bone fixation and healing.

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Radiopaque Magnesium Phosphate Cements

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INTRODUCTION: Magnesium phosphate cements (MPC) are attracting growing interest in the field of clinical applications, not only due to their high strength and fast setting reaction¹ but also for the potential role that Mg can play as an essential element in the turnover of mineralized tissues. Moreover, previous studies have revealed good antimicrobial properties for some MPC formulations [1]. Several clinical applications require radiopaque materials in order to track them by X-ray imaging. Therefore, the goal of this work was to develop MPC formulations with high level of radiopacity.

METHODS: Three formulations of MPC, based on MgO and either ammonium dihydrogen phosphate (NH₄H₂PO₄), sodium dihydrogen phosphate (NaH₂PO₄) or a mixture of both were prepared. The formulations were coded as NH₄-MPC, Na-MPC or NH₄+Na-MPC, and were generally designated as MPC. MPCs containing bismuth oxide (Bi₂O₃) as radiopacifying agent were also prepared², and were named as rad-MPC. The exothermy of the two families (MPC and rad-MPC) was adjusted by the addition of borax as a retardant. The cement powder was mixed with distilled water, in a liquid to powder ratio of 0.13 ml/g.

The effect of Bi₂O₃ on different properties of the cements was evaluated. The setting times were measured with Gilmore needles. The injectability of the cement paste was evaluated by the instrumented extrusion in a Universal Testing Machine through a 2 mm-aperture syringe, up to a maximum force of 100 N. The phase composition was assessed by XRD. Finally, the compressive strength after different setting periods was evaluated in a Universal Testing Machine, and the morphology and total porosity of the cement were assessed by Field Emission Scanning Electron Microscopy and Mercury Intrusion Porosimetry, respectively.

RESULTS: Adequate radiopacity [2] of rad-MPC was achieved with 10wt% Bi₂O₃. The addition of Bi₂O₃ resulted in a reduction of the exothermy of the setting reaction, rad-MPCs

requiring a smaller amount of retardant (1 wt% of borax, in front of the 3% required for the MPCs).

In general, rad-MPCs had shorter setting times (between 6 and 11 min for the three formulations) than their MPC counterparts (between 8 and 15 min), which was associated with the lower amount of retardant added in rad-MPCs. As a general trend, the addition of the radiopacifier resulted in an increase of the injectability of MPC pastes.

The final products of the three MPC formulations were not affected by the addition of the radiopacifying agent. NH₄-MPC resulted in a mixture of struvite and schertelite, and Na-MPC in an amorphous phase, irrespective of the Bi₂O₃ content. However, the hardening of the rad-MPCs was slower than their MPC counterparts, although the maximum compressive strength attained was within the same range (42-48 MPa). The addition of Bi₂O₃ reduced the total porosity of the cements, from 20% for the MPCs to 5% for the rad-MPCs.

DISCUSSION & CONCLUSIONS: MPCs with adequate radiopacity and setting exothermy were obtained by the addition of Bi₂O₃ in the cement powder, without compromising the setting properties, the injectability and the maximum strength of the cements. This represents a significant advantage for some specific clinical applications that require a precise tracking of the material during or after implantation.

REFERENCES: ¹ Mestres G et al. Acta Biomater, 2011, 7:1853-1861; ² UNE-EN ISO 9917-1:2007.

ACKNOWLEDGEMENTS: This work was supported through Project MAT2009-13547.

Nanostructure of injectable hydrogel composites

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INTRODUCTION: Tissue replacement materials are of immense value to medicine and a topic of widespread research interest. Hyaluronic acid hydrogels are one of the appealing materials that could function as matrix to incorporate organic or inorganic substances to enhance tissue growth or act as a delivery system. This work involves the characterization of a hyaluronic acid based scaffold that has shown good biocompatibility and degradability properties within the field of bone regeneration [1]. Using small angle neutron scattering (SANS) one can obtain a scattering profile of an inhomogeneous and fragile hydrogel composite system, which will determine the correlation length inside the gel structure as well as the fractal aggregation of the particles in the scaffold.

METHODS: Gels of different concentrations were made by cross-linking the aldehyde-modified hyaluronic acid with hydrazide-modified polyvinyl alcohol. SANS measurements were performed on the pure gels and gels incorporated with nano-sized hydroxyapatite particles [2].

RESULTS: The scattering curves showed two distinctive regions which indicate two different length scales in the gel system, see Figure 1.

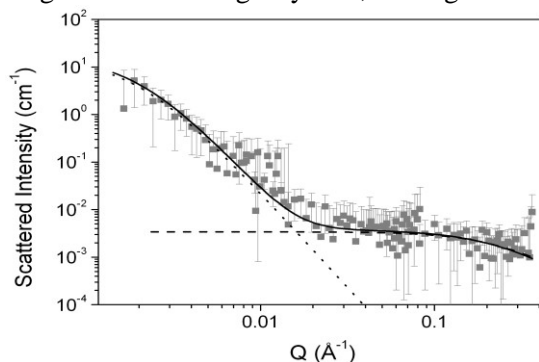


Fig. 1: Scattering profile model shown as the solid line. The dotted and the dashed lines show the two components of the fit.

A pictorial view of the inhomogeneities in the gel network is shown in Figure 2. The two correlation lengths, ζ and Ξ , describe the mesh

size of the gel and the average distance between polymer rich regions. The fitted results are shown in Table 1.

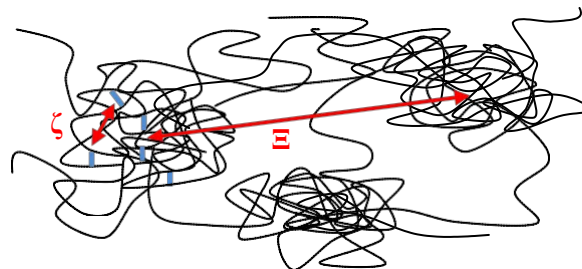


Fig. 2: Pictorial view of the gel.

Table 1. Correlation length for the 5% and 15% gels.

Sample	ζ (Å)	Ξ (Å)
5% gel	18±3	250±10
15% gel	21±3	220±10

Scattering profiles of the 15% gel samples containing 5, 10 or 20 wt% particles could be superimposed, which suggests that the overall aggregation structure of particles is not concentration dependent. Data analyzed by a cylindrical and mass fractal model showed that the particles with a radius of ~ 100 Å and a height of ~ 30 Å formed clusters with radius of gyration >1000 Å.

DISCUSSION & CONCLUSIONS: SANS allowed us to understand the previously poorly characterized hydrogel system. We established a model system which describes the gel with two length scales which is beyond the traditional picture of a cross-linked hydrogel. We were able to determine the two correlation lengths inside the inhomogeneous gel as well as aggregation profile of mass fractal aggregates. This work forms part of a wider project that attempts to understand material properties at a molecular level and apply this knowledge to biomaterial sciences.

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Mechanical properties of a ceramic bone substitute loaded with Gentamicin

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INTRODUCTION: In order to prevent bone infections after surgery, an antibiotic loaded bone substitute could be used. Such a material is the bioactive and injectable ceramic filler Cerament™ loaded with Gentamicin. Cerament™ is biphasic, consisting of 60wt% calcium sulphate and 40wt% hydroxyapatite, which make it partly resorbable. In vivo the calcium sulphate component is resorbed with time, the hydroxyapatite remains intact providing an osteoconductive support for in-growth of new bone [1], and the Gentamicin is released. Gentamicin is an aminoglycoside antibiotic, acting by inhibiting protein synthesis at the 30S subunit of the ribosome of the bacteria [2]. The advantage with using Cerament™ as a delivery vehicle is that the device both acts as a bone substitute promoting bone healing and prevents colonization of microorganisms. Cerament™ does not need to be surgically removed, as PMMA beads do which normally are used to prevent infection. The purpose of this investigation was to characterize the mechanical properties of Cerament™ loaded with Gentamicin.

METHODS:

The material used in this study was kits with 18.5g Cerament™ powder (consisting of 60wt% calcium sulphate hemihydrate and 40wt% hydroxyapatite) and 380mg Gentamicin sulphate (GS) that was dissolved with 8mL saline. The saline-GS solution was injected into the powder in a specially designed mixing- and injection device. Mixing was conducted for 30s, before the paste was transferred to a 10mL syringe and used for mechanical tests¹.

The following mechanical tests were performed: *injectability time frame* (how long time it was possible to extrude paste through a 16G cannula connected to a 10mL syringe with force $\leq 150\text{N}$), *moldability time frame* (time frame when a paste heap could be molded without being too smeary and not too dry), *dry setting time* (Gillmore needles ASTM C266-08, at ambient temperature), *wet setting time*

(Gillmore needles; 37°C Ringer solution), *dry compressive strength* (rods of 4mm in diameter and 8mm in length; dried at 40°C before being compressed with 1mm/min), *wet compressive strength* (after 24h in 37°C Ringer solution), and *unmolding time of hard beads* (time point when hardened beads, in a bead mold, could be unmolded). All measurements were performed three times.

RESULTS: Results are presented in table 1.

Table 1. Mechanical properties of Cerament™ loaded with Gentamicin.

Test	Results (mean \pm std)
Injectability time frame	Possible to inject until: T $< 5.8 \pm 0.6\text{min}$
Moldability time frame	Start time: $5.8 \pm 0.3\text{min}$ Stop time: $7.6 \pm 0.1\text{min}$
Dry setting time	IST = $8.5 \pm 0.4\text{min}$ FST = $10.4 \pm 0.3\text{min}$
Wet setting time (37°C)	IST = $10.5 \pm 0.4\text{min}$ FST = $13.2 \pm 0.8\text{min}$
Dry compressive strength	$42.1 \pm 9.3\text{MPa}$
Wet compressive strength	$10.6 \pm 1.9\text{MPa}$
Unmolding time of hard beads	T = $10.7 \pm 0.6\text{min}$

DISCUSSION & CONCLUSIONS: The Gentamicin loaded ceramic bone substitute Cerament™ could be injected, molded and used for preparation of beads. Interestingly, FST is reached very close to IST. Clinically this means that the patient can be mobilized very soon after the surgeon is done with the material injection and the wound had been closed. The mechanical properties are promising for use in non load-bearing applications.

Elution studies and animal studies are currently on-going.

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¹ The final bone substitute paste contained approx 16.5mg Gentamicin / mL paste.

Polymers influencing the strength of injectable calcium phosphate bone cements – a preliminary study

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INTRODUCTION: Due to their similarities to bone mineral, calcium phosphate cements (CPC) are used as replacement for autograft bone. Depending on the phase of the set cement CPCs can be either apatite or brushite. Apatite cements are more investigated but, an increasing amount of research is conducted on brushite cements since they can be resorbed faster than apatite *in vivo*. Numerous brushite cement compositions have been investigated to improve the setting, injection, and mechanical properties [1]. To improve the brittleness of CPCs, polymers have been incorporated in the cement. The affect of poly(acrylic acid) (PAA) on the strength of apatite cements [2] has been investigated and some improved mechanical properties could be seen. To our knowledge this has not been made for brushite cements. In this study it was investigated if PAA can be used in brushite cements. It was also investigated if poly(acrylic acid-co-ethylene glycol methyl ether acrylate) (P(AA-co-EGMEA)), which is more hydrophobic than PAA, and poly(styrene sulfonic acid) (PSS), which has a lower pK_a than PAA, could be used.

METHODS: Conventional brushite cements; monocalcium phosphate monohydrate and beta-tricalcium phosphate in a 1:1 ratio were used as ceramic precursor powder. To increase the setting time, 1wt% of sodium pyrophosphate was added to the powder mixture. The polymers used were polymerized in bulk through radical polymerization initiated by azobisisobutyronitrile, and were dissolved in water in 2.5w/w% after purification. The liquid to powder ratio (L/P) were set to 0.4 mL/g. Cylindrical moulds, 6 x 12 mm, were filled with paste and left to cure for 24 hours in 37°C. The compressive strength (CS) was measured using a universal testing machine (Shimadzu AGS-X).

RESULTS: All cements set within less than one hour. Comparing the cements containing polymers both P(AA-co-EGMEA) as well as PSS had higher strengths than PAA (*Table 1*). However, it can be noted that all cements were weaker than the water based control. During

mixing, the cements containing polymers got a gel-like consistency that kept together well, compared to than the water based control.

Table 1. CS of investigated cements, standard deviation within brackets

Polymer	CS (MPa)
PAA	2.6 (0.2)
P(AA-co-EGMEA)	3.5 (0.2)
PSS	4.0 (0.5)
Only water	5.1 (0.7)

DISCUSSION & CONCLUSIONS: The preliminary results presented here show that it is possible to use acid polymers in the acidic brushite cements, but that the compressive strength is decreased. Results also indicate that increasing the hydrophobicity of the polymer increases the strength. Furthermore, the lower pK_a of PSS might positively affect the strength. It has previously been shown that carboxylic acids attract free Ca^{2+} , preventing the ions from participating in the brushite reaction and thus resulting in a calcium deficient, weak cement. In addition, alpha-hydroxyl carboxylic acids attract the Ca^{2+} on the growing brushite surface, slowing down the crystallization process, giving a stronger cement [3]. The results presented in our study indicate that the polymers used have a mechanism similar to carboxylic acids without any alfa-hydroxyl group and create a calcium-deficient weaker cement. Future studies should therefore concentrate on increasing the calcium concentration in the powder phase and investigation of polymers containing alfa-hydroxyl acids, both carboxylic and sulfonic. To conclude; the incorporation of polymers to brushite cement is possible, but other polymers need to be investigated to possibly improve the strength.

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Synthesis of β -TCP platelets for bone substitutes

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INTRODUCTION: Calcium phosphate (CaP) ceramics are widely used as bone graft substitutes [1]. β -tricalcium phosphate (β -TCP) is of particular interest because it is biocompatible, has a chemistry close to that of the mineral part of bone and is actively resorbed by osteoclasts [2]. β -TCP is a phase that is usually obtained by a high temperature process [1]. Hence, the particles are agglomerated, have undefined shapes and wide size distributions, limiting their potential use, especially as reinforcement in composite structures or in injectable materials. Recently, the synthesis of hexagonal β -TCP single crystals by precipitation in ethylene glycol (EG) at a relatively low temperature (150°C) was reported [3]. The effects of temperature and concentration on crystal morphology and crystallinity were briefly investigated, but no attention was paid on crystal size (diameter, d , and thickness, h) and aspect ratio ($s=d/h$). The aim of this study was to understand more of the β -TCP crystallization process and to find ways to tune the size and aspect ratio of the particles. In particular, the kinetics of the reaction was studied for one composition and the influence of the concentration of Ca^{2+} and PO_4^{3-} ions and of Mg^{2+} ions was investigated.

METHODS: A 22.6mM Na_2HPO_4 ethylene glycol solution was added to a 13.6mM CaCl_2 ethylene glycol solution at 150°C (with a Ca/P molar ratio of 1.5). After 24h under intense stirring, the solution was cooled down in air and rinsed using centrifugation steps in ethanol and demineralised water. The crystalline composition was determined by XRD and the size and aspect ratio of the particles were measured by image analysis of SEM images. The kinetics of the reaction was studied by taking out a few mL of the solution at regular intervals. Those samples were rapidly cooled down in ethanol at room temperature and centrifuged. Beside the kinetics study, the concentration of Ca^{2+} and PO_4^{3-} ions was varied from 0.95 to 19mM and from 0.634 to 12.68mM, respectively (keeping a constant Ca/P molar ratio=1.5) and the amount of Mg^{2+} ions was varied between 0 and 1 mol%.

RESULTS & DISCUSSION: During the first seconds of the reaction, only a gel-looking phase was observed, possibly amorphous CaP [3]. A few small hexagonal platelets were observed from 30s ($d=200\text{-}300\text{nm}$, $h=60\text{-}75\text{nm}$) (Fig. 1a). According to XRD, these platelets consisted of β -TCP. The size of the hexagons increased linearly with time ($\partial d/\partial t = 7\text{nm/s}$ and $\partial h/\partial t = 0.8\text{nm/s}$), reaching $d=600\text{-}850\text{nm}$ and $h=125\text{-}170\text{nm}$ at 2min (Fig. 1b). Later, the amorphous phase disappeared completely (Fig. 1c), but the size did not increase significantly anymore ($\partial d/\partial t$ and $\partial h/\partial t < 0.001\text{nm/s}$). The size and aspect ratio of the platelets can thus not be increased indefinitely with the reaction time.

The size and aspect ratio of the particles increased with increasing Ca^{2+} and PO_4^{3-} ions concentration but remained below $d=1\mu\text{m}$ and $s=8$.

The presence of Mg^{2+} ions in the solution clearly decreased the size and aspect ratio of the crystals. Without addition of Mg^{2+} ions, the crystals were $\sim 800\text{nm}$ wide and $\sim 150\text{nm}$ thick. Their size decreased linearly down to $d\approx 130\text{nm}$ and $h\approx 44\text{nm}$ with 1mol% of Mg ions. This emphasises the need for Mg-free chemicals if large particles with a high aspect ratio are desired. Indeed, magnesium ions are often present as impurities in CaCl_2 .

In all conditions, the platelets size dispersion was very narrow ($\text{SD}/\text{mean} < 0.10$, see Fig. 1c).

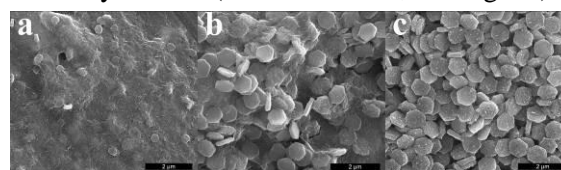


Fig. 1: Evolution of crystallization with time. (a) 50s, (b) 2min, (c) 32h. The scale bar is $2\mu\text{m}$.

CONCLUSIONS: The reaction time, the presence of impurities (Mg^{2+}) or the concentration of calcium and phosphate ions influence the size and aspect ratio of β -TCP platelets, but these effects are limited.

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Trauma fracture augmentation: Can fracture severity indicate outcome?

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INTRODUCTION: There have been limited studies on the use of vertebroplasty for traumatic spinal fracture repair. To date, there are no validated biomechanical models available to compare between augmentation materials over short-cycle, high-load cyclic loading. The aim of this study was to investigate behaviour over such loading and determine whether the severity of the fracture plays a role in the success of the augmentation.

METHODS: Due to the scarcity of young cadaveric tissue and the similarities with human vertebrae [1], 48 three-vertebra functional spinal units (FSU) were harvested from the thoracolumbar region of 12 porcine spines. The FSUs were fractured using a previously developed method [2], which produced traumatic fractures in 22 vertebrae. Three sets were devised: a control set of n=12 non-augmented specimens (NONE), n=7 augmented with laboratory grade polymethylmetacrylate (PMMA) and n=6 augmented with a calcium phosphate cement (CaP) [3]. Specimens were augmented using a bi-pedicular technique and were subsequently housed between two parallel plates of PMMA to facilitate loading prior to being imaged using a micro computed tomography (μ CT) system.

The resulting images were graded three times using an adaptation of a previously established method [4] and the average was taken to give an indication towards fracture severity (Fig. 1).

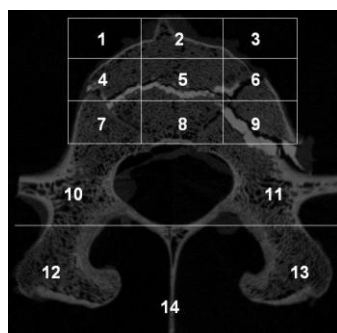


Fig. 1: The adapted grading schematic⁴

The specimens were loaded in compression using a materials testing machine (10 kN Instron 3366, UK) to a pre-load of 1 kN (10

mm/min) after which they were loaded and unloaded 30 times from 1 kN to 6 kN (25 mm/min). The stiffness of each specimen during each loading was obtained.

RESULTS: A total of n=12 specimens underwent the entire loading regime. In all cases, the stiffness increased over the cycles, indicating that the load caused cumulative compressive damage to the specimens (Fig. 2). Lower fracture severity grade and higher initial stiffness were better indicators of survival than the augmentation set, with both augmented and non-augmented specimens reaching the full 30 cycles.

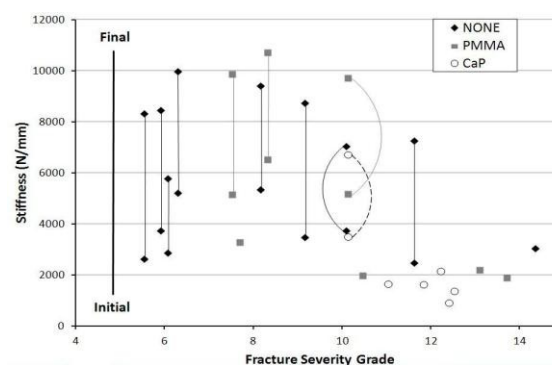


Fig. 2: The relationship between fracture severity, stiffness and augmentation (note axis).

DISCUSSION & CONCLUSIONS: A high fracture severity score could be used clinically as a contraindication for vertebroplasty, since it appears augmentation alone is not sufficient to maintain stability in these cases. In biomechanical testing, initial stiffness and fracture severity grade could be used to reduce error associated with fracture variability.

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Drug delivery carriers and injectable biomaterials simultaneously

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INTRODUCTION: This presentation will describe the possibility with injectable biomaterials, and at the same time the use of these biomaterials as carriers for delivery of

attractive for chemically bonded bioceramics. Special attention will be paid the CaO-Al₂O₃-

hydrated phases, and inert porous additives.

METHODS: Studies were executed by evaluating the chemical reactions and microstructure using HRTEM, XRD, XPS and STEM with EDX [2]. The mechanical properties including compressive and flexural strength, Young's modulus and fracture toughness were evaluated [3]. The Ca-aluminate based materials have been evaluated comprehensively concerning their biocompatibility and toxicological endpoints, the harmonized standard ISO 10993:2003. *In vitro* bioactivity was evaluated using methods described in ISO standard 23317, and chemical resistance in acid environment using the water jet impinging test, EN 29917:1994/ISO 9917:1991.

RESULTS: High-resolution TEM and BET surface area analyses reveal the micro-structure to consist of hydrates having a size of 10-50 nm and nano-size channels located between said hydrates of a width of 1-2 nm. The development of microstructures includes different type of porosity, amount of porosity, pore size and pore channel size, and combination of different porosity structures. Complementary porosity above 5-10 nm is obtained by partial hydration of the precursor material, excess of water in the hydration step or additional porosity of high element inert ceramic selected in order to increase strength and radio-opacity.

The control of the porosity is highly related to 1) the cement phases, 2) the particle size of the cements, 3) the hydration temperature, 4) the environment (complementary ions), 5) processing agents, 6) inert additives, 7) the volume involved, and 8) the compact density and the w/c ratio. Summary in Fig.1.

Log (pore size in nm)

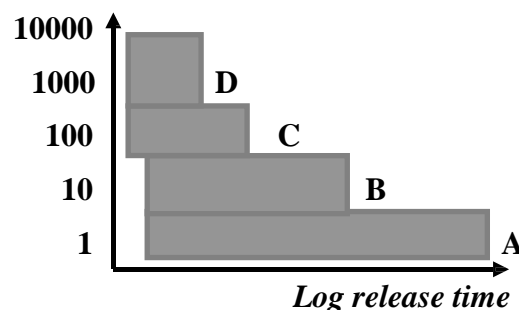


Fig. 1: Pore size interval for different types of ceramics (A = Fully hydrated, B = Partially hydrated, C = Sintered ceramics (submicron), D = Sintered ceramics (partially or coarse grain)).

DISCUSSION & CONCLUSIONS: The property profile of the Ca-aluminate materials, the processing (control of hydration) and specifically their microstructure, make these biomaterials potential also as carriers for controlled drug delivery.

The carrier material can be applied as a solid or a suspension for sublingual or oral intake, subcutaneous or percutaneous injection. The drug carrier can also work as an injectable implant.

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Cytotoxicity and Mechanical Properties of Castor Oil Modified PMMA Bone Cements

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INTRODUCTION: To avoid adverse biomechanical effects close to reinforced vertebra, low modulus bone cements are desired in vertebroplasty [1]. To reduce the stiffness of poly(methyl methacrylate) (PMMA) bone cements, the use of plasticizers, e.g. castor oil, seems to be a promising approach [2]. However, the cytotoxicity of such materials due to leakage of harmful components still needs to be resolved. In this context, it also has to be noted that there is a lack of appropriate testing procedures for the cytotoxicity of injectable biomaterials, specifically addressing their curing nature. The aim of this study was to develop PMMA based bone cements with adequate mechanical properties and biocompatibility, and to establish a testing protocol that would reveal the time dependent cytotoxic effects of the curing materials.

METHODS: Different concentrations of castor oil (Sigma-Aldrich) were incorporated into Simplex™ P (Stryker) bone cements (with additional radiopacifier) by replacing equivalent volumes of the liquid monomer. Mechanical properties of the resulting cements were assessed according to ASTM F451. Extracts of cements were obtained by adding cell culture medium to the curing cements 2.5min after commencement of the mixing. Extraction was carried out at 37°C. The extraction media were replaced after 1h, 12h and 24h. Cells from the osteoblastic cell lines MG-63 and SAOS-2 were cultured in 24 well plates. After 24h the culture medium was replaced by the different extraction media. Cell viability was assessed at specific time points using the alamarBlue® viability assay.

RESULTS: Both the Young's modulus and the maximum compressive strength were found to decrease with increasing concentrations of castor oil. Cements containing 12% castor oil had a modulus of 480 MPa and maintained an adequate strength of 25 MPa. The cytotoxic potential of the extraction media from the

individual cements varied for the cell types used in this study. MG-63 cells showed lower cell viability after 1 day of culture in the different extracts. In contrast, SAOS-2 cells were less sensitive to the extraction media (Fig. 1). Furthermore, for both cell types the addition of castor oil had only minor effects on the cytotoxicity of the cement extracts. However, there was a trend that different oil concentrations changed the time dependent release of toxic components from the materials.

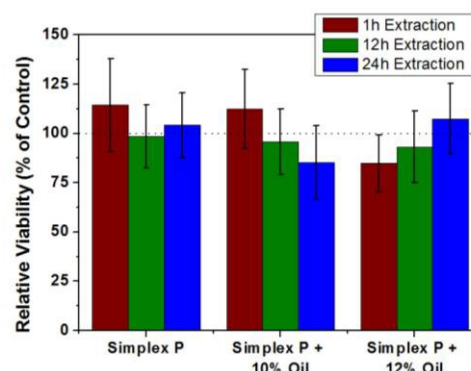


Fig. 1: Viability of SAOS-2 cells after 1 day of culture in extraction media from unmodified and oil modified Simplex™ P bone cements.

DISCUSSION & CONCLUSIONS: Using castor oil as an additive in PMMA bone cements is a promising approach towards low modulus materials for vertebroplasty. Compared to unmodified cements, the addition of the oil resulted in lower Young's moduli without considerably altering their cytotoxicity. However, the experiments revealed that the chosen cell types as well as the extraction conditions play a key role during the in vitro evaluation of biological responses to curing, injectable bone cements.

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ACKNOWLEDGEMENTS: Funding from the European Union (VPHOP FP7-ICT2008-223865 and SPINEGO PERG07-GA-2010-268134) as well as VINNOVA (VINNMER 2010-02073) is gratefully acknowledged.

Effects of polysaccharides on the injectability of a mineral bone cement

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INTRODUCTION: Self-setting mineral cements were developed over the last thirty years for bone filling and reconstruction in orthopedics and dentistry. One interesting property is their paste moldability which makes them possibly injectable. While it limits surgery trauma and infection risks, their implantation using an injection system is often associated with a phase separation (“filter-pressing”). [1] Several additives such as polysaccharides – to prevent from filter-pressing – and silver – to confer antibacterial activity – can be introduced into cement liquid and/or solid phases.

The aim of this study is to determine how the incorporation of silver-loaded spray-dried microspheres or commercial powder of polysaccharides (carboxymethyl cellulose (CMC) or carrageenan (CRG)) into the cement solid phase formulation affects the injectability of the cement paste.

METHODS: The cements were prepared by mixing the solid phase (S) consisting of a mixture of equal weight of vaterite (CaCO₃) and dicalcium phosphate dihydrate (DCPD; CaHPO₄, 2H₂O) with an aqueous liquid phase (L) using L/S = 0.7. [2] Two kinds of composite cements were prepared by adding to the mineral solid phase 2 wt% or 10 wt% of CMC or CRG introduced as a powder (*p*-CMC and *p*-CRG) or Ag-loaded microspheres (*μ*-CMC and *μ*-CRG). The latter were synthesized by spray drying. Injectability tests were performed using a texturometer measuring the charge needed to extrude at a constant rate a volume of cement paste from a syringe. [3] The cement microstructure and composition were investigated during and after extrusion and setting at 37°C using FTIR spectroscopy, X-ray diffraction (XRD) and scanning electron microscopy (SEM).

RESULTS: The results showed that introduction of CMC or CRG led to a significant improvement of the injectability of the paste and suppressed filter-pressing. Whatever the type, the form and the amount of polysaccharide introduced into the solid phase,

the cohesiveness of the paste was improved and the composite was injectable. For the 2% polysaccharide-loaded cement, the charge needed for paste extrusion varied from *ca* 6 kg for 2%-*μ*-CMC and 2%-*p*-CRG to *ca* 4.5 kg for 2%-*p*-CMC. In addition, it remained constant during the extrusion of the *μ*-composites whereas it slightly decreased for the *p*-composites. Interestingly, the injectability of 10% polysaccharide-loaded composites was even better and more reproducible (3.5-4.5 kg).

DISCUSSION & CONCLUSIONS: Several studies have been reported on the introduction of polysaccharides only into cement liquid phase to improve injectability. [4] We observed that with CMC or CRG into the solid phase, the cement paste seems to lose its shear thinning behavior and to be more elastic and cohesive. We showed that the presence of polysaccharide promoted a strong orientation of all DCPD platelets during extrusion: their biggest faces were parallel to the direction of extrusion, thus decreasing paste filter-pressing. We can assume that water interacting with polysaccharide has a determinant role on the paste injectability and cohesiveness: partial dissolution of the polysaccharide could contribute in controlling viscosity and/or lubricating effect in the paste. The feasibility of injectable self-setting composites including polysaccharide into the solid phase has been demonstrated and a rheological study is in progress for a better characterization of the composite paste behavior. In addition, the antibacterial activity and biofilm formation capability of such Ag-loaded composites is under investigation.

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Hydrogels for BMP-2 delivery: Influence of carrier nature and pH on ectopic bone formation

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INTRODUCTION: Bone morphogenetic protein-2 (BMP-2) may be used in bone repair to circumvent limitations of autologous bone grafting. Therapeutic BMP-2 application requires a carefully designed delivery carrier to prevent rapid clearance from the application site and preserve protein function. A potential pitfall lies in rhBMP-2 bioactivity loss due to conformation and aggregation changes close to the physiological pH [1]. Two biopolymers are attractive carrier options: hyaluronic acid (HY) and chitosan (CH). The objective of the present work is to compare the osteoinductive activity of rhBMP-2-loaded CH and HY carriers in a rat ectopic bone induction model at two different pH [2].

METHODS: A new injectable chitosan hydrogel that forms *in situ* a biodegradable gel [3] and a commercial cross-linked hyaluronan were used. Hydrogels loaded with 145 µg rhBMP-2 (Inductos®, Wyeth Pharmaceuticals) were prepared at pH 4.8±0.2 and 6.2±0.2. CH and HY hydrogels, loaded or not with rhBMP-2, were injected in rat quadriceps of Sprague-Dawley rats (n=6). Mineralized bone volume (MBV) was assessed at 3 weeks by microCT-scan and histopathology. Paired Wilcoxon test at p<0.05 level were used for analysis.

RESULTS: Bone formation was observed at 3 weeks with both carrier types at both pH values.

Controls devoid of rhBMP-2 did not induce bone. Higher bone formation was observed at low pH (4.8) compared to high pH (6.2), with ratio $MBV_{low\ pH}/MBV_{high\ pH}$ significantly higher than unity. HY hydrogel demonstrated a significantly higher bone formation compared to the CH hydrogel. Histopathological analysis demonstrated both trabecular and woven bone surrounding a hematopoietic bone marrow, with congestive vessels. No remains of injected chitosan hydrogel were detected. In contrast, hyaluronan hydrogel was not completely resorbed.

DISCUSSION & CONCLUSIONS:

The present study highlights the importance of the carrier's characteristics such as formulation pH and nature for osteoinductive activity of rhBMP-2. The rhBMP-2 bioactivity decreased at high pH probably due to protein aggregation and/or conformational changes [1]. The higher rhBMP-2 bioactivity in HY hydrogel might be explained by higher protein retention at the injection site due to the ionic complexation of the protein with HY and slower resorption of HY hydrogel compared to CH hydrogel.

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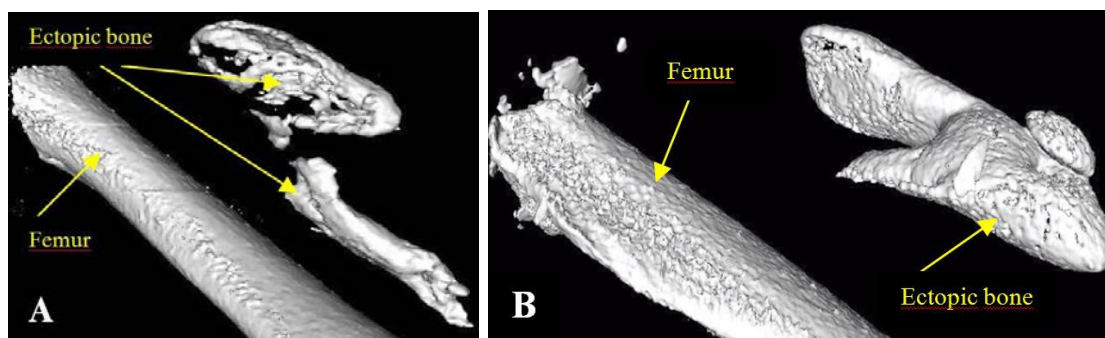


Fig. 1: CT-scan surface rendering showing ectopic bone at 3 weeks induced by 150 µg rhBMP-2 in chitosan (A) and hyaluronic acid (B) hydrogels at pH 4.8

Interpretation of calorimetric measurements on an α -TCP/ α -CSH cement

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INTRODUCTION: Isothermal calorimetry can be used to track the evolution of exothermic processes in real-time. The reaction of a biphasic bone substitute of α -TCP (α -tricalcium phosphate) and α -CSH (α -calcium sulfate hemihydrate) was monitored by isothermal calorimetry. The two solid phases convert into calcium-deficient hydroxyapatite (CDHA) and calcium sulfate dihydrate (CSD), respectively. The thermal signature of hydrating cements can provide insight into their mechanism of hydration. However, many different approaches exist in the literature concerning the treatment of calorimetric data and their interpretation.

METHODS: Commercial α -TCP and α -CSH powders, combined with Na_2HPO_4 , were mixed into a paste by using a water based X-ray contrast medium containing iohexol (300 mgI / ml). 6 g of powder with 96.5 wt% of an 80/20 α -TCP/ α -CSH mix and 3.5 wt% Na_2HPO_4 were hand-mixed with the liquid phase to a liquid/powder ratio of 0.45 ml/g. The mixing was made at room temperature for 0.5–1 min and the samples were then charged into a TAM Air isothermal conduction calorimeter (Thermometric AB) at 37 °C.

RESULTS: Isothermal calorimetry measurements of the hydration of the α -TCP/ α -CSH cement revealed three thermal events (a, b, c), as shown in Fig 1. The true kinetics of the heat production rate were calculated with the Tian equation [1].

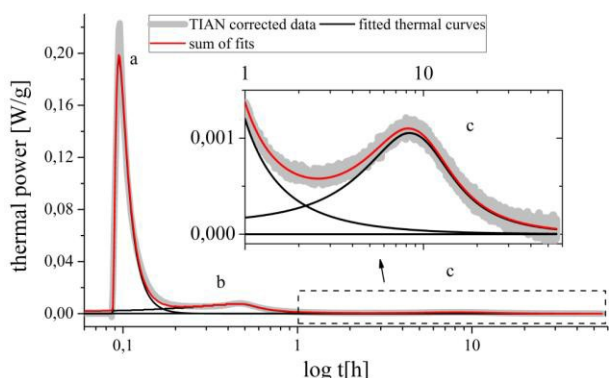


Fig. 1: Example of results from calorimetric measurements.

A curve fitting software (fityk 0.9.7) was used to separate the result into three separate events. Many experimental runs have been performed to study the heat contributions from the two solid phases, but here only basic treatment of the data is discussed and the information that can be obtained from this type of measurements.

DISCUSSION & CONCLUSIONS: The treatment of calorimetric data is different among the studies of α -TCP-based cements found in the literature. Although the Tian-correction is described as a necessary step [1] it is not used in some studies [2, 3]. Additionally, the first thermal event (a) is often labelled as a wetting peak [3, 4], but is in some cases entirely omitted from kinetic analyses [4]. It is often not clear whether event “a” actually contains any hydration information or if it is only an artefact of the method. Some studies show plots with calculated degrees of α -TCP conversion, based only on the calorimetric data, without considering that the reaction may be incomplete due to a limited amount of added fluid [2]. Normalizing to different amounts of total emitted heat from various cement compositions will lead to incorrect comparisons if the reactions are incomplete. An additional point arises from the fitting of the calorimetric data so that the different thermal events can be separated; can this provide information about the reaction mechanism? We conclude that it is important to revisit calorimetric data as they are basic to understanding the reaction by which bone cements of this type set.

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Stem Cell-Calcium phosphate Scaffolds for Bone Engineering

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INTRODUCTION: Seven million people suffer bone fractures in the US each year [1, 2], and musculoskeletal conditions cost \$215 billion annually [1]. These numbers are predicted to increase rapidly as the population ages. Allografts and xenografts raise concerns of immunorejection and disease transmission. Recent advances in tissue engineering have led to the development of new materials and strategies offering immense promise for these patients. The introduction of stem cells into the clinical settings opens new horizons. New injectable calcium phosphate scaffolds with the ability to deliver cells and bioactive factors in minimally invasive surgeries make attractive alternatives to the current conventional treatments. The aim of this study was to 1- develop injectable, mechanically strong titanate- loaded calcium phosphate scaffolds, characterize the physicochemical and biological properties of developed scaffolds. 2- evaluate the cytotoxicity of human bone marrow mesenchymal stem cells (hBMSCs) encapsulated *in vitro* 3- investigating the effects of CPC-titanate scaffold on the adhesion, proliferation and differentiation of the hBMSCs.

METHODS: Cement powder was combined with either polymethylvinyl ether maleic acid or polyacrylic acid and ceramic titanate nanoparticles to obtain Type I and Type II scaffolds respectively. Commercial injectable calcium phosphate cement was selected as control. Phase composition was examined by x-ray diffraction. Setting time, injectability, compressive and diametral strengths were measured and compared with the control. Set scaffolds were placed in cell culture with (hBMSCs). Cellular function, alkaline phosphatase activity (ALP) and osteogenic differentiation were assessed.

RESULTS: X-ray diffraction patterns of Type I and Type II scaffolds showed hydroxyapatite. Setting time was 5-15 minutes. The scaffolds showed superior injectability, significantly higher compressive and diametral strengths

values compared to commercial cement. Percentage of live (hBMSCs) attaching to scaffolds increased to 99% at 14 days. Cells proliferated to $(1808 \pm 317 \text{ cells/mm}^2)$ at 14 days.

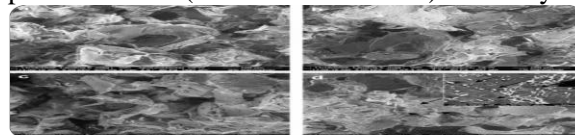


Fig. 1: Scanning electron microscopy of titanate –loaded calcium phosphate scaffolds

Table 1. Mean compressive strength and standard deviation of control cement and the two calcium phosphate scaffolds (CPCs)

Cement	1h	24h	1w	2w
Control	1.90 ±0.30	8.0 ±0.90	13.4 ±2.1	17.4 ±2.9
Type I	40.4 ±3.25	71.7 ±2.80	79.5 ±3.65	67.1 ±4.12
Type II	86 ±2.9	90.5 ±4.0	78.6 ±4.22	73.6 ±3.10

DISCUSSION & CONCLUSIONS: The incorporation of titanate nanoparticles into Type I and II scaffolds imparted high strength values, injectability, biocompatibility, and suitable setting times. hBMSCs proliferation matched that of the control. hBMSCs on the scaffolds differentiated down the osteogenic lineage and expressed high levels of bone marker (ALP). Type I and II scaffolds may be useful for stem cell-based regeneration in maxillofacial and orthopaedic applications.

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ACKNOWLEDGEMENTS: We thank Dr. ElZoghby for discussions and experimental assistance.

Investigation of injectable Hyaluronan-Iron-oxide hybrid nanogels as dual imaging agents

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INTRODUCTION: Imaging and cellular uptake of new hybrid materials present an exciting avenue to explore new treatment strategies for diseases such as arthritis, bone regeneration and cancer [1, 2]. In this study, we propose to investigate the role of iron oxide based hybrid nanogels linked to both low and high molecular weight hyaluronic acid (HA)-bisphosphonates and additionally functionalized with a fluorescent tracker. These nanogels would be tested for biocompatibility and the kinetics of uptake and sub-cellular localization will be probed in a GFP-fibroblast cell line. HA is known to have specific interactions with the CD44 receptor amongst others. Previous cellular studies have indicated the specificity of the functionalized HA in receptor mediated endocytosis through this type of interaction. The novelty of the study lies in the fact that we have developed a dual-imaging strategy that could be exploited simultaneously *in vitro* as well as *in vivo* uptake studies.

METHODS:

Preparation of Nanogels: Hyaluronic acid was acquired from Lifecore Biomedical. The functionalized HA-bisphosphonates materials of low and high molecular weight were mixed with dilute and varying concentrations of FeCl₂ and FeCl₃ solutions. By modulating the pH conditions, nanogels containing HA-hy-BP-Fe₃O₄ were formed. These hybrid nanogels were further labelled with Rhodamine Red to enhance its functionality as a cell tracker.

Imaging of Cells: Cellular uptake is proposed using confocal microscopy. GFP-labelled rat dermal fibroblasts will be seeded at a density of 5x10⁵ cells/mL in chambered cover glass slides (Nunc, Fisher-Sci). Cells will be left overnight at 37°C to promote adhesion. Cells will be incubated with HA-hy-BP-Fe₃O₄ nanogels (50 ug/mL) in serum free media for varying times. Cells will be washed with PBS and will be treated with LysoTracker-594 DND (Invitrogen) at a final concentration of 1 μM,

fixed and then treated with DAPI (300 μM). Samples will be processed for imaging under a confocal microscope.

MRI of Cells: Cells will be prepared and seeded as before in a 6 well plate. Cells will be treated with the HA-hy-BP-Fe₃O₄ nanogels ((100 μg/mL, 10 μg/ml Fe) in separate well with serum free media and incubated for 0.25, 0.5, 1, 2, 3 and 24 h, respectively at 37°C. Cells will be washed and imaged with an MRI machine.

RESULTS: We have prepared HA-hy-BP-Fe₃O₄ nanogels that are around 120nm. These particles have been conjugated with Rhodamine Red. Cytotoxicity and imaging experiments are on going and we expect the results soon.

DISCUSSION & CONCLUSIONS: Our hypothesis aims to provide a dual imaging platform that can be utilized for *in vitro* targeting and localization events and to predict possible mechanisms of uptake, while the same particles can be used for looking at bio-distribution and homing capabilities when injected intravenously or via a catheter. This increases the possibilities of using the vehicles to carry drugs like bisphosphonates that are used to treat bone resorption to using them to deliver anti-cancer drugs. The particles being biodegradable would be cleared from the system eventually.

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ACKNOWLEDGEMENTS: This work is supported generously by the EU-MultiTERM project (FP-6, 2009-2013).

A new method for characterizing the viscoelastic deformation behaviour of hydraulic bone cements

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INTRODUCTION: It is important to find methods to characterize injectable bone cements to understand how they work during application. The objective of this study was to find a method which describes the properties of hydraulic bone cements during deformation. Two calcium sulphate based materials with different consistency were examined.

METHODS: The two materials (Cement 1 and Cement 2) used in this study were two calcium sulphate based injectable bone cements (60wt% calcium sulphate, 40wt% hydroxyapatite, added to an iodine containing liquid) with similar particle size and setting behaviour. However Cement 2 contained an organic viscosity enhancing agent which gave Cement 1 and Cement 2 clearly different consistencies (obvious when touching the pastes).

Initially the two cements were characterized rheologically using a method based on an ASTM standard (steady state oscillation), which describes the dynamic mechanical properties [1]. The measurements were done with an Anton Paar, MCR301 rheometer between 2 and 18 minutes at a constant strain of 0.05%. Then the two cements were characterized by using a specially designed High Strain Oscillation method which simulates the deformation of the material during application. The measurements were performed between 2 and 16 minutes at a constant strain of 20%.

RESULTS: The “ASTM- method” showed no difference between Cement 1 and Cement 2. It only captured the setting behaviour and because the two cements have a similar setting behaviour and similar structure building process no difference could be seen in the results. The different consistency of the two cements was not shown with this method. The High Strain Oscillation method showed in comparison to that that Cement 1 and Cement 2 react differently on high deformations. Cement 1 has a higher complex viscosity than Cement 2 during deformation. This means that Cement 1

is more robust during deformation intervals and can withstand higher deformations (see Fig 1).

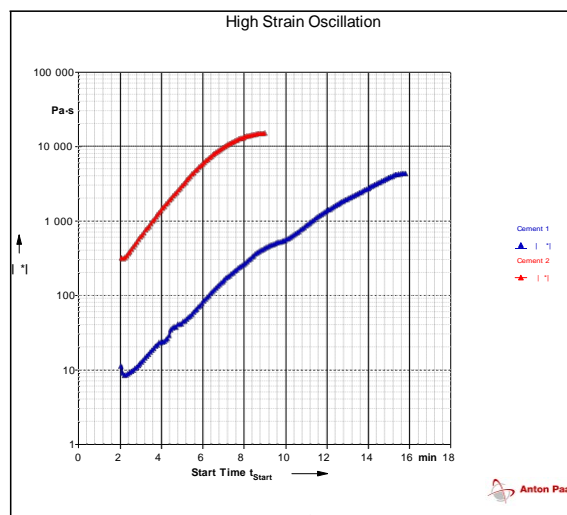


Fig.1: High Strain Oscillation for Cement 1 and 2

DISCUSSION & CONCLUSIONS: This study has shown that the new High Strain Oscillation method gives promising results and captures the difference between Cement 1 and 2 which could be observed in the consistency but not measured earlier. Cement 1 and Cement 2 have different consistencies and different viscoelastic deformation behaviour. In this case they cannot handle deformation in the same way. To summarize the high strain oscillation method is a promising method for characterizing hydraulic bone cements.

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Thermo-mechanical coupled FE-Simulation of Acrylic Bone Cement Curing after Vertebroplasty

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INTRODUCTION: Vertebroplasty is a surgical method to treat osteoporotic damaged or fractured vertebral bodies. Within this treatment a biomaterial (bone cement) gets injected into the vertebral body in liquid state, cures to a solid in vivo and thus leads to a stabilisation of the vertebrae. Especially acrylic bone cements are widely used as biomaterial to be injected. The curing of this class of materials is induced by an exothermal chemical reaction and therefore leads to heat generation and a rising temperature within the vertebral body. In order to describe the thermo-mechanical coupled curing process within the human body, a material model for acrylic bone cements has already been developed [1] and will be applied to finite element (FE-) models of human vertebral bodies.

METHODS: The geometric models of trabecular bone are produced out of μ CT scans of human vertebral bodies. An identification of representative boundaries between the bone material and other human tissue is realised and the boundary is converted to an approximated surface using triangulation. With the aid of this triangulated surface an automated FE-mesh of the trabecular bone region as well as the ambient volume is generated. The latter one is used to describe the marrow and the bone cement. Furthermore, the same geometric model is used to simulate the injection process of acrylic bone cement by CFD-simulations. This gives information about the distribution of bone cement within the vertebrae as well as other physical data, e.g. the temperature distribution and the progress of the curing process. These results are utilised to define the regions of bone cement and the remaining human tissue to the FE-mesh. Moreover, the local results are assigned to the integration points of the FE-mesh. By this approach, the initial conditions of the FE-simulation are

defined. The material behaviour of the acrylic bone cement is described by the developed material model [1] and material models for the trabecular bone and the marrow are based on literature data [2].

RESULTS: The procedure of FE-modelling and simulation is demonstrated using a reduced test example, which represents FE-mesh of an extract of $3 \times 3 \times 3 \text{ mm}^3$ of human trabecular bone of the vertebrae (cf. Figure 1, the illustration of the marrow mesh is suppressed).

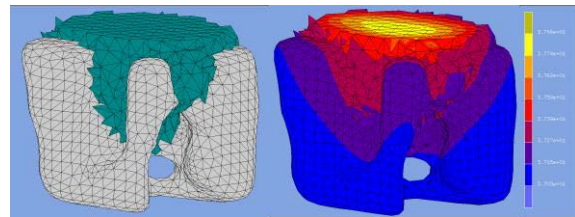


Fig. 1: FE-Mesh of bone and bone cement (left) and computed temperature field (right)

The depicted temperature distribution is caused only due to the exothermal curing process of the acrylic bone cement.

DISCUSSION & CONCLUSIONS: By applying the developed material for acrylic bone cements in conjunction with the described FE-modelling procedure, a unified approach for the analysis of thermo-mechanical processes in vertebroplasty is achieved. In future work, more complex geometries as well as different discretisation techniques will be surveyed. Furthermore, the impact of different initial cement distributions and varying process parameters on the characteristics of treated vertebral bodies will be analysed.

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Laponite/alginate hydrogels for release of doxorubicin - an injectable pH sensitive hybrid material

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INTRODUCTION: Alginate (AG) hydrogels are being widely studied as carriers for drug release or encapsulation of cells, due to easy drug loading and simple administration procedures [1]. However, great challenge still exists concerning how to confer them stimuli-responsive properties and improve their drug encapsulation efficiency (*EE*) and sustained release ability [2]. Laponite (LP) (25 nm in diameter and 1 nm in thickness) can establish strong interactions with guest compounds [3]. In our previous work [3, 4], the incorporation of LP into AG hydrogels greatly improved the drug encapsulation efficiency of methylene blue (MB), a cationic hydrophilic model drug, and the pH sensibility in MB release. To explore the potential application of LP/AG gels, in this report, novel pH-sensitive hybrid hydrogels was investigated for delivery of a real anticancer drug, doxorubicin (DOX), by incorporating LP into Ca²⁺-crosslinked AG hydrogels.

METHODS: The LP/AG beads containing DOX were prepared by dropping 5 mL aqueous solutions of LP/AG/DOX (x/1/0.2 (wt.%), x = 0 and 0.2) into 50 mL of 0.05 M/0.05 M CaCl₂/CaSO₄ solution. After, the beads were washed with distilled water thrice (total 100 mL) and dried under vacuum at room temperature or in an oven at 40 °C to get vacuum-dried gels (H-v) (0-v and 0.2-v) and oven-dried gels (H-o) (0-o and 0.2-o), respectively. The *EE* and *in vitro* drug release were spectrophotometrically determined at 475 nm. The swelling behaviors of the hydrogels were determined *in vitro* by mass measurements (one bead /0.2 mL PBS).

RESULTS: Increasing LP content from 0 to 0.2 wt.%, the *EE* of DOX in AG gels increased from 87.9 to 98.5 wt.%. The half-release time (*t*_{1/2}) of DOX for H-o increased from ~5.5 h (0-o) to ~37 h (0.2-o), while *t*_{1/2} for H-v changed from ~2.5 h (0-v) to ~25.5 h (0.2-v) at pH 7.4 (Fig.1). Compared to the pure AG gels, the hybrid beads showed more pH sensibility in both drug release rate and drug release

efficiency. The incorporation of LP, the variation of the drying mode and the pH value caused a change in the mechanisms of DOX release.

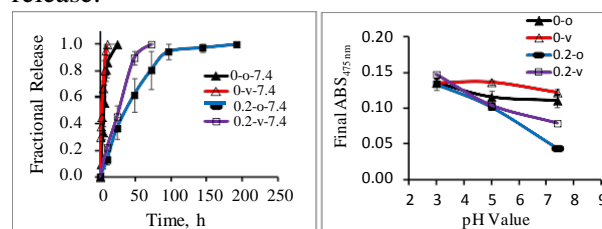


Fig. 1: Effect of LP on the drug release rate in PBS at pH 7.4 (left), and pH on the drug release efficiency (right) for AG and its hybrid gels with LP at different drying modes.

DISCUSSION & CONCLUSIONS: The addition of LP, combined with oven drying, not only improved the drug encapsulation efficiency of DOX in AG gels, but also the pH sensitivity of the DOX release efficiency and drug release rate, through changing the drug release mechanisms. The systems could be useful for localized delivery of cationic anticancer drugs, for tumor treatment (pH 5-6.8) [4].

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Strontium Halides as Degradable Radiopacifiers

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INTRODUCTION: Current investigations on calcium phosphate cements seek to improve their performance for use in somewhat more demanding applications. Their use in e.g. the spine would require them to be radiopaque for adequate control during the surgical procedure. Radiopacifiers such as barium sulphate (BaSO₄) and zirconium dioxide (ZrO₂) are commonly included in acrylic formulations; however, these do not degrade *in vivo* and could compromise the compressive strength of the already fragile calcium phosphates [1]. Most strontium halides are water-soluble compounds that can release strontium ions, which have the additional advantage of stimulating the osteoblasts and reducing the activity of the osteoclasts [2]. This study assessed the effect of four strontium halides on the radiopacity, mechanical strength, and cytotoxicity of brushite cements.

METHODS: Brushite cements were prepared by dissolving monocalcium phosphate monohydrate (MCPM) and di-sodium dihydrogen pyrophosphate in distilled water. Upon dissolution, the β -tricalcium phosphate (β -TCP) and the radiopacifier (5 to 20 wt%) were incorporated. The following compounds were tested: strontium fluoride (SrF₂), strontium chloride hexahydrate (SrCl₂·6H₂O), strontium bromide (SrBr₂), and strontium iodide (SrI₂). The total powder-to-liquid ratio was 3.3 g/mL, the molar ratio MCPM: β -TCP was 1:1. Specimens of 1 mm thickness were irradiated with 72 kV_p and the radiopacity was calculated relative to a standard aluminium ladder (1 to 5 mm Al). The compressive strength of the cements was also investigated. In addition, *in vitro* cell viability was assessed using human osteoblast like cells, Saos-2, which were seeded on the cement specimens and analyzed by live/dead stain after 1 and 3 days.

RESULTS: The radiopacity increased with both the concentration and the atomic number of the corresponding halide resulting in the SrI₂-containing brushites (*Fig.1*) to be the most radiopaque materials. Moreover, the strength did not significantly change with the concentration of radiopacifier although it was generally higher for SrI₂-containing specimens

and concentrations between 5 and 15 wt%. Interestingly, the addition of 10 wt% SrBr₂ and SrI₂ improved the cell viability after 3 days.

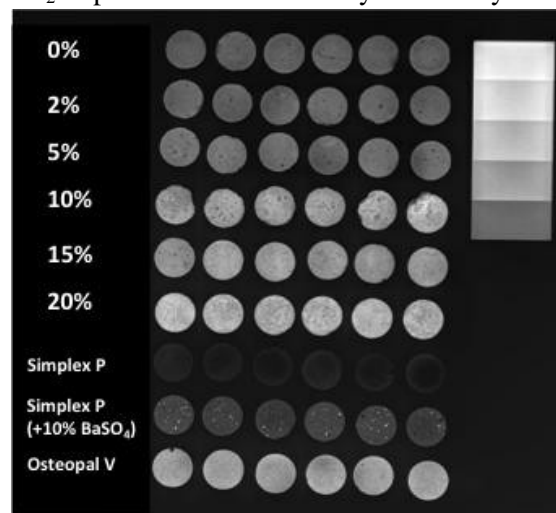


Fig. 1: Selected radiography of SrI₂-containing (2 to 20 wt%) brushite cements in contrast with commercial PMMA cements.

DISCUSSION & CONCLUSIONS: Osteopal V (Heraeus Medical) is an acrylic cement designed for vertebroplasty that contains 45 wt% ZrO₂; Simplex P (Stryker) is designed for prosthesis fixation and contains only 10 wt% BaSO₄ but is sometimes used for vertebroplasty with additional BaSO₄. The results showed that the addition of 10 to 20 wt% SrBr₂ or SrI₂ to calcium phosphate formulations gives brushite cements with similar radiopacity to that of Osteopal V, and much higher radiopacity than that of Simplex P with additional 10 to 30 wt% BaSO₄. It was shown that adding 10 to 15 wt% SrBr₂ or SrI₂ slightly improves the strength and the cell viability after 3 days, respect to standard brushite and to those modified with SrCl₂·6H₂O and SrF₂ as radiopaque agents.

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Bioactivity of porous PMMA-CaP cements.

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INTRODUCTION: Polymethylmethacrylate (PMMA) was the first type of cement used in spine applications. PMMA is inert and porous materials are better for anchoring purposes [1]. Adding calcium phosphates (CaPs) to porous PMMA intends to establish a balance between the mechanical and the biological properties provided by these two materials [2]. This work aims to study the bioactivity of porous PMMA cements loaded with CaPs.

METHODS: Porous PMMA consisted of a commercially available PMMA cement kit (Palavit® 55VS; Heraeus), water, carboxymethylcellulose (CMC, Akucell, AkzoNobel) and CaP powders, *i.e.* 85% alpha tricalcium phosphate (CAM Implants BV), 10% dicalcium phosphate anhydrous (J.T. Baker Chemical Co) and 5% precipitated hydroxyapatite (Merck). Porous PMMA were obtained by using the procedure developed by de Wijn [3]. Briefly, 2.1 g of PMMA were mixed with 0.19 g of CMC. This was subsequently mixed with 1 ml of MMA until obtaining a homogeneous paste. Next, 2.8 ml of water were added and everything was mixed again. Then, the CaP powders were added and mixed in order to load the PMMA paste with CaPs. Amounts of 0.048, 0.095, 0.19, 0.38 and 0.76 g of CaPs were loaded. Pristine porous PMMA, *i.e.* without CaPs, was the control. Cement pastes were mold into cylinders with dimensions of 6 mm in diameter by 12 mm in height. Samples were immersed in a calcium physiological solution (CPS) -at 37 °C- for 0, 3, 7, 14, 21 and 28 days to evaluate their bioactivity. Scanning electron microscopy (SEM; JEOL 6310), X-Ray diffraction (XRD; PW3710 Philips) and Fourier-transform infrared spectroscopy (FTIR; Spectrum One Perkin Elmer) characterized the bioactivity of the porous PMMA samples in terms of morphology, compounds present and chemical composition, respectively. Micro Computed Tomography (μ -CT, SkyScan 1072, SkyScan) characterized their structure.

RESULTS: SEM showed that the CaP powders were well incorporated in the porous PMMA and that both materials interacted with the CPS. This was corroborated by XRD, FTIR. As the percentage of loaded CaPs increased its detection by XRD and FTIR was easier. μ -CT described similar structural properties for all porous PMMA samples.

DISCUSSION AND CONCLUSION: This study showed that loading porous PMMA with CaPs was feasible. CaPs were loaded into the CMC phase. This allowed generating a porous PMMA with a more soluble phase which was loaded with CaPs. This could enhance the osteoconductive and bonding properties of these porous PMMA materials to bony sites by providing them with CaPs [4]. The interaction of the CaP powders with the CPS was possible and signaled the presence of an open porous structure. CaP loading did not affect the porous PMMA; FTIR did not detect an interference of the loaded CaPs with the PMMA. The results obtained in this study make the porous PMMA cement loaded with CaPs an interesting material. However, more information about mechanical and porometrical properties, as well as biological *in vitro* and *in vivo* studies are needed to fully validate the candidature of these materials for spine applications.

ACKNOWLEDGEMENTS: The Smart Mix Program of the Netherlands Ministry of Economic Affairs and the Netherlands Ministry of Education, Culture and Science is acknowledged for its financial support. The NCMLS, St. Radboud, is acknowledged for the SEM facilities.

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Preliminary studies on an injectable polymeric biomaterial based on renewable resources

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INTRODUCTION: During the last couple of decades the use of renewable resources has increased significantly as an alternative to the limited deposits of oil, coal and gas. Starch as an example of “green” raw material has become a backbone for a great number of new chemicals that either have interesting properties or may replace traditional compounds often identified as toxic. It is worth of mentioning that currently used biomaterials like bone cements are based on monomers of petrochemical origin. Many of those substances are suspected of toxicity or have been identified as harmful to human health. This paper includes preliminary studies on synthesis and further characterisation of end-use properties after curing of novel dimethacrylate based on derived from starch human friendly – isosorbide.

METHODS: The novel dimethacrylate based on isosorbide derived from human friendly starch - 2,5-bis(2-hydroxy-3-methacryloyloxypropoxy)-1,4:3,6-dianhydro-sorbitol (ISDGMA) was obtained in a three-step synthesis shown in fig. 1. Product obtained was identified and characterised by means of NMR and IR spectroscopy as well as ESI-MS spectrometry. Subsequently, compositions of novel monomer (0.7 – 0.9 w/w) with triethylene glycol dimethacrylate (TEGDMA) were prepared and used in formulation of model *in-situ* curable bone cement with 0.3 w/w mineral filler (biphasic calcium phosphate). Monomer compositions and model cements were cured at 25 °C using benzoyl peroxide/*N,N'*-dimethyl-*p*-toluidine initiating system. Cylinder-like samples were used to determine compressive properties. Those measurements were repeated after a week of conditioning in simulated physiological environment i.e. phosphate buffer solution (pH = 7.4) at 37 °C. Water sorption (7 days) was determined as well.

RESULTS: The novel dimethacrylate based on bioderived alicyclic diol – isosorbide was obtained with overall yield of 80 %. Structure of resulting monomer was confirmed by means of complementary analytical methods. Results of compression testing are presented in tab. 1. Significant decrease of compression modulus and yield point is observed after a week of conditioning, while the ultimate compressive strength is not significantly different. Determined water sorption ranges from 7.5 % to 9.8 % in case of filled materials and 9.6 % to 13.1 % for unfilled ones.

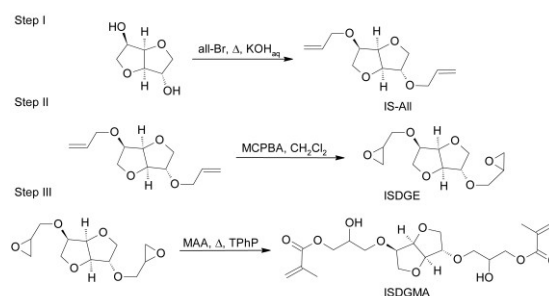


Fig. 1: Scheme of the three-step synthesis of ISDGMA.

Table 1. Comparison of compressive properties of model bone cement compositions (results after conditioning in brackets).

Property	Modulus [MPa]	Yield [MPa]	Strength [MPa]
Material	2545-2879	74.1-78.2	123.1-156.7
	(1078-1456)	(26.2-34.7)	(104.1-169.7)

DISCUSSION & CONCLUSIONS: In spite of a strong dependence of mechanical properties on water sorption, novel materials show a potential in substitution of selected hard tissues. It has been shown that the model injectable dimethacrylic bone cement based on the bioderived monomer might become an interesting alternative to currently used *in-situ* curable biomaterials.

Local delivery of Simvastatin from calcium phosphate cement

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INTRODUCTION: Locally applied simvastatin (SV) is known to stimulate bone formation [1, 2]. However, the lack of suitable delivery systems has restricted its clinical use. In the present study a novel approach of using calcium phosphate cement (CPC) as potential drug carrier for simvastatin was evaluated. A release study was performed to measure the release kinetics. In addition, an *in vitro* study with human osteosarcoma cell line MG-63 was done to assess the effect of SV on bone formation.

METHODS: The calcium phosphate cement used in this study consisted of monocalcium phosphate anhydrous, β -tricalcium phosphate and glycerol [3]. The two powder components were mixed and glycerol was added to obtain a paste.

Simvastatin was mixed with the glycerol and added to the cement to get an end dose of 0.5 mg SV/g cement paste. To evaluate the release of the drug, cement samples were moulded and immersed in PBS, pH 7.4, at 37 °C under continuous shaking. Every 24 hours, samples were collected and replaced with fresh PBS to approximate sink conditions. The amount of drug released was determined spectrophotometrically at a wavelength of 238 nm.

The biological effects of SV were tested using the osteoblastic cell line MG-63. The cells were cultured in 96 well plates at a density of 10000 cells/cm². The cells were let to adhere for 4h. Different concentrations of SV, in the same concentration ranges as obtained in the release study, were added to the culture medium. This was done in order to mimic the drug release from the cement. The medium was changed every three days. After 8 days of culturing, the cells were fixed with ethanol and stained with alizarin red, which stains the calcium produced by the cells. Alizarin red stain could therefore be used as a measure of bone cell differentiation.

RESULTS: The release profile showed that there was a burst release of SV from the cement during the first day. However, the remaining

SV was released in a more sustained manner for over a week. Light microscopical investigations revealed a less dense cell culture in the wells treated with 10⁻⁶ M SV compared to the control. In contrast, the cell density was higher than the control in wells treated with 10⁻⁷ M SV. The alizarin red stain was also more intense in the wells with 10⁻⁷ M SV compared to the control (Fig. 1).

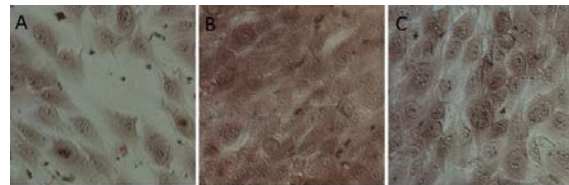


Fig. 1: Alizarin red stained MG-63 cells after 8 days of cultivation with different concentrations of SV. A) 10⁻⁶ M SV B) 10⁻⁷ M SV and C) control with only cell medium.

DISCUSSION & CONCLUSIONS: The results show that the particular CPC evaluated in this study is a promising delivery system for SV. The material has the ability of slowly releasing the drug for more than one week. Furthermore, the results from the alizarin red staining (Fig. 1) suggests that low concentrations of SV both stimulates bone formation and cell proliferation. To confirm and quantify the proliferation data observed in this study further quantitative proliferation assays needs to be done. Conclusively, the addition of small amounts of SV into CPC could add osteogenic properties to the cement. However, the amount of SV loaded has to be adjusted to fit the therapeutic range of stimulating bone formation.

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The effect of mechanical properties of the carrier materials on BMP-2 delivery and ectopic bone formation

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INTRODUCTION: A group of injectable hyaluronan-based hydrogels have been previously suggested as a potent delivery vehicles for bone morphogenetic protein 2 (BMP-2) for bone induction purposes. [1] In this work we investigate the implications varying the setting time prior to injection on the mechanical properties of hydrogels and consequently on the BMP-2 delivery and bone formation.

METHODS: Hydrogels loaded with BMP-2 (InductOs, Pfizer) and hydroxyapatite were prepared through chemical crosslinking of an aldehyde functionalized hyaluronan and hydrazide functionalized poly(vinyl alcohol). A hydrogel premix was prepared by mixing the two polymer components back and forth in syringes interconnected by the tip. The premix left to cure in one of the syringes for 1 min, 5 h or 3 d, prior to being injected. This resulted in the formation of slightly, moderately and fully cured hydrogels at the point of injection. The 1 min, 5 h or 3 d hydrogels were compared by studying the release of radiolabeled BMP-2 in vitro from each respective scaffold. The hydrogels were also evaluated in vivo in a subcutaneous ectopic model in male Sprague–Dawley rats.

RESULTS: Similar release profiles are observed for all three test groups in vitro. The analysis of the explanted grafts showed cancellous bone formation in all groups after 5 weeks in vivo. However, longer pre-incubation times give rise to a higher bone volume. Furthermore, the 5 h and the 3 d grafts appeared to be more ordered and resistant to deformation from the surrounding tissue than the 1 min grafts. Moreover, in the 3 d group formation of cartilage was observed, suggesting that in this group bone formed via the endochondral ossification pathway, rather than via the intramembranous ossification pathway (*Fig. 1*).

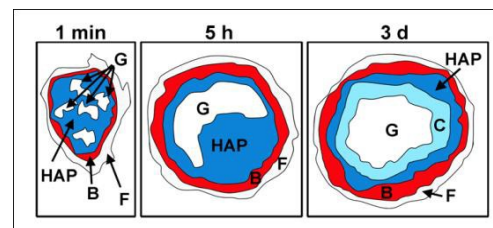


Fig. 1: A summary of the morphological differences in the 1 min, 5 h and 3 d grafts after 5 weeks in vivo: cartilage (C), connective tissue (F), gel (G) and hydroxyapatite (HAP) residues.

DISCUSSION & CONCLUSIONS: The results of this study suggest that variation of incubation of the hydrogel premix has direct implication on the stability of the hydrogel at the time of injection. Injection shortly after the initiation of the crosslinking results in less mechanically stable hydrogels, while longer pre-incubation gives rise to more rigid materials, but risks decrease in BMP-2 stability, which could explain the presence of endochondral ossification. In our opinion the obtained information may be used to tune the mechanical properties of the material to match specific applications, where high bone volume or material compliance may be of interest.

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Release of Gentamicin from a ceramic bone substitute

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INTRODUCTION: In order to prevent bone infections due to surgery, antibiotic loaded bone substitutes could be used. The rate and extent of antibiotic release from such bone substitutes are dependent on several factors including the type of antibiotics incorporated and the properties of the bone substitute. For PMMA based materials, for example, the antibiotic release is often seen to be dependent on the surface area of the implant [1].

The aim of the study was to examine if there were any differences in the release of Gentamicin (G) from a Gentamicin sulfate (GS) loaded ceramic bone substitute (Cerament™) with different surface/volume ratios and if the material was used as pre-set beads or as an injectable paste.

METHODS: The ceramic material used in this study was 18.5g Cerament™ powder (consisting of 60 wt% calcium sulfate hemihydrate and 40 wt% hydroxyapatite), 380 mg GS and 8mL saline solution. The samples were prepared by dissolving the 380 mg GS powder in 8 mL saline and thereafter mix the GS/saline suspension with the Cerament™ powder for 30 seconds in a specially designed mixing- and injection device. The prepared GS loaded Cerament™ pastes were used to prepare three groups of samples (“LS”, “HS” and “P”). All as triplicates.

In two of the groups *pre-set materials* were prepared with the same total volume and mass of the paste (10 mL which corresponds to about 19 g). The group “LS” (Low Surface) consisted of pre-set material with a low surface (~ 24 cm²), whereas the group “HS” (High Surface) were made of 6 mm beads (>100 cm²). 50 mL Ringer solution (37 °C) was added to the pre-set materials and the samples were placed in an incubator at 37 °C.

The third group consisted of an *injectable paste* (“P”) with the same volume, mass and surface area as “LS”, but in this case the material had not set before it was extruded into the 50 ml Ringer solution (37 °C).

The G concentrations in the Ringer solution surrounding the different samples were investigated for up to 8 days. The model used for the sample outtake assumed no infection and that 10 ml bone substitute was surrounded by 50 ml tissue and that 20 % of this volume was exchanged daily. Therefore, 10 ml of the Ringer solution in all the samples were replaced every 24 h. The G concentration in the solution taken out was analyzed using CEDIA (Cloned Enzyme Donor Immuno Assay).

RESULTS: Figure 1 shows that the sample with the highest surface (HS) initially contributed to a higher G concentration, but after 3 days, this difference cannot be seen any longer. No significant difference in G concentration can be seen depending on if the Ringer solution and the GS loaded Cerament™ come in contact before or after hardening.

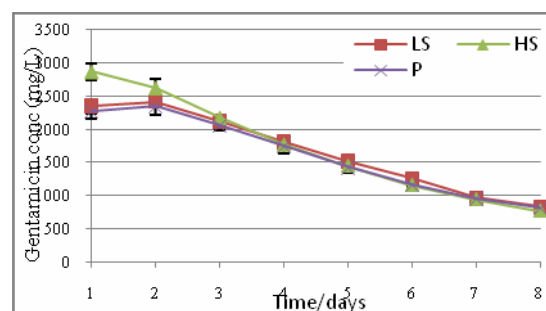


Fig 1: Gentamicin concentration in the Ringer solution surrounding the LS, HS and P samples.

DISCUSSION & CONCLUSIONS: The release profile of Gentamicin from the ceramic bone substitute Cerament™ is similar, no matter if the material has been used as pre-set beads (with different surface area) or as an injected paste.

Comparative elution trials with GS loaded PMMA as well as animal studies with GS loaded Cerament™ are currently on-going.

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ACKNOWLEDGEMENTS: Klinisk Kemi, Universitetssjukhuset, Lund

Adhesion to dental hard tissues and ethanol wet bonding

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INTRODUCTION: The adhesion to dental hard tissues relies on a number of liquid products presented in syringe form, pen or small squeezable bottle forms in order to deliver active compounds to a precise location. Basically, the first etching step involves removal of the smear layer and surface demineralization of the dentine in order to expose clean collagen fibrils. The hydrated collagen fibrils resulting from the water rinsing step to remove excess acid and reaction products are then soaked in a hydrophilic monomer such as hydroxyethylmethacrylate. This monomer is intended to replace water and infiltrate the spaces left void by dissolution of the dentinal hydroxyapatite (HA). However, the composite layer obtained by polymerization of monomers does not reach the depth of the demineralization front, leaving exposed collagen fibrils not fully embedded in resin. Self-etch products combining an acid and monomer functional groups are an attempt to resolve the observed hiatus between sound dentine and tooth restorations. If the molecule responsible for demineralization is not washed away but polymerized in situ, then no gap should be left between dentine and resin. But clinical results often show a decrease in adhesion strength with time, attributed to collagen hydrolysis adjacent to the hybrid layer. This is due to the presence of water solvent needed for acid expression and due to the hydrophilic nature of the polymer resin formed which causes water uptake. This is inevitable even with acid monomers forming insoluble calcium salts or binding directly to HA surfaces [1]. Following decalcification and water rinsing, an ethanol rinse has been proposed in order to promote the penetration of more hydrophobic monomers further inside the demineralized dentine [2].

METHODS: We provide physico-chemical evidence, using ATG, DSC, NMR and I.R. techniques, that several types of water molecules are present in demineralized collagen. Histologic sections were observed.

RESULTS: We show that with the ethanol rinse, the final water content of the polymerized hybrid layer reduces to the amount (ca 6%) originally present in sound dentin. DSC shows endothermic peaks at 0°C for bulk water, and other peaks for bonded water down to -20°C. Collagen denaturation occurs between 65 and 95°C. Microscopic observations reveal a more homogeneous hybrid layer with ethanol rinse.

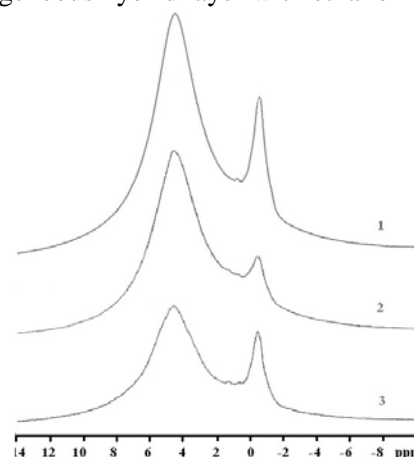


Fig. 1: Proton MAS NMR of demin.dentin ethanol washed(1), then with adhesive (2), with adhesive but without ethanol(3).

Table 1. Amount of water present in dentine.

Sample	Weight % water loss
Untreated dentine powder	6.4
Demin dentine	33.8
Demin dentine + EtOH	15.3
Demin dentine + adhesive	10.5
Demin dentine +EtOH + adhesive	5.3

DISCUSSION & CONCLUSIONS: Ethanol wet bonding helps in making a stable dentin-restoration interface by water minimization. This residual water is un-freezable, is strongly bound to the collagen fibrils hydrogen bond network and may contribute neither to its denaturation by heat, nor to its hydrolysis.

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Mineralization of thermosensitive chitosan hydrogels by sorption of Ca from CaCl₂ solution for biomedical applications

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INTRODUCTION: Chitosan is a natural polysaccharide derived by deacetylation of chitin, a component of the exoskeletons of crustaceans and has been widely applied as a biomaterial (drug delivery systems and scaffolds for tissue engineering) due to its properties including biocompatibility with the human body, non-toxicity, biodegradability and ability to form gels at physiological pH and temperature. Thermosensitive chitosan hydrogels were formed by neutralization of acidic chitosan chloride solution using sodium beta-glycerophosphate (β -GP). For medical applications in bone regeneration, however, the presence of a mineral phase based on calcium and phosphorus would lead to increasing osteoconductivity. Additionally porous surfaces are known to promote differentiation of cells (osteoblasts) and promote osteogenic differentiation. As chitosan/ β -GP hydrogels have particular sorption capacity mineralization was induced by their incubation in CaCl₂ aqueous solution acting as a source for sorption of calcium. Therefore, the aim of the current study was to investigate the mechanism of that mineralization.

METHODS: Chitosan/ β -GP injectable gels were produced according to a protocol derived from Chenite et al. [1]. Briefly, 16ml of 2.5% (w/v) chitosan in 0.1 M HCl was mixed with 2ml of 1g/ml (β -GP) solution in water. Their gelation was induced by increasing temperature close to natural body temperature. Mineralized hybrid system was induced by adsorption of Ca from different concentrations of Ca in solutions of CaCl₂ after gel formation (post-loading) and then optionally to increase phosphorus amount in the structure, before lyophilization, hydrogels were incubated in phosphate buffer at pH=7 or pH=6.

RESULTS: The structure and mineralization effect of the chitosan gels was studied by analyzing molecular structure (FTIR) sample 1gCa/dm³ pH7 spectra revealed that apatitic

phosphate bands became more pronounced in the region around 1030 cm⁻¹ and 850 cm⁻¹ Figure 1. Rheological measurements confirm gelation point appear at 37°C.

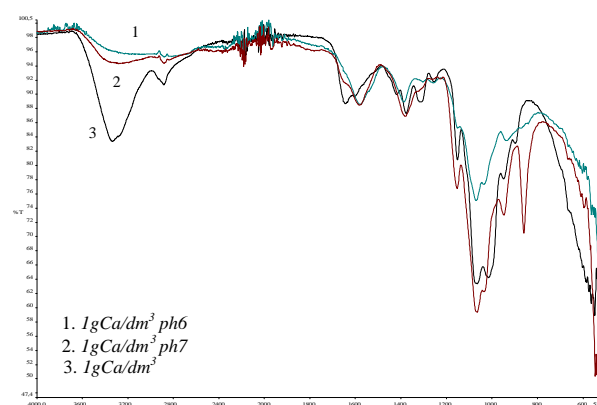


Fig. 1: FTIR spectra

DISCUSSION & CONCLUSIONS: It can be concluded that all studies indicate the presence of Ca and P in the scaffolds structure at ratio Ca/P=1.0 was obtained by sorption mechanism and it could be an interesting material for tissue engineering.

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Bone Formation Performance of Reindeer Bone Protein Extract Formulations, Autograft and Demineralized Bone Matrix in Sheep Hole Defect Model

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INTRODUCTION: Autograft is the traditional method in bone repair enhancement, but harvesting of bone grafts can lead to complications¹. The good bone forming and healing capacity of the reindeer bone protein extract and its various formulations have been demonstrated in small animal models. The aim of this study was to compare beta tricalcium phosphate (β -TCP)-carriers in a stearic acid – modified Polyethylene Glycol/Glycerol (PEG-GLY) matrix with or without reindeer bone protein extract using a sheep hole defect model. Bone autograft, commercially available demineralized bone matrix (DBM), pure β -TCP-granules and empty defects were used as controls.

METHODS: Hole defects 6 mm in diameter and 10 mm in depth were induced to the femoral and humeral distal and proximal condyles of sheep hind and front legs with a drill under general anaesthesia². The holes were filled with the different test materials or left empty. Resorption of carrier, amount formation and bone remodelling and carrier resorption in and quality of new bone and general bone healing were evaluated from defects by micro-CT analysis after eight weeks follow-up.

RESULTS: The micro-CT analysis showed good bone the groups with the bone protein extract. In the groups with pure β -TCP or formulated β -TCP granules with no protein extract, the resorption of granules was not seen and the bone formation was typically around the granules. The bone formation in the groups with the reindeer bone protein extract was comparable to autograft. The least bone formation was observed in the DBM and untreated groups. (Fig.1).

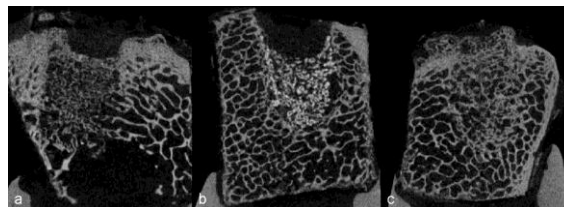


Fig. 1: Resorption of carrier, amount and quality of new bone were evaluated using micro-CT analysis. a) Paste with reindeer bone protein extract, b) Controlled paste without protein extract, c) Autograft.

DISCUSSION & CONCLUSIONS: The results showed that the reindeer bone protein extract with β -TCP-carrier was as good as autograft in the bone forming and defect healing. This was an encouraging result when finding a substitute method for the autograft treatment.

In conclusion, the β -TCP-granules in the PEG-GLY matrix with stearic acid is a functional scaffold system for the reindeer bone protein extract but proportional amount and form of granules in matrix must be yet optimised. The reindeer bone protein extract and β -TCP formulation in the paste form is the suitable alternative for autograft.

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ACKNOWLEDGEMENTS: The authors would like to thank to BBS - Bioactive Bone Substitutes Ltd for providing the reindeer bone extract and samples. This study was partly supported by the Academy of Finland, National Doctoral Programme of Musculoskeletal Disorders and Biomaterials (TBDP), the Ahti Pekkala Foundation, and the University of Oulu Support Foundation (Kerttu Saalasti Foundation).

Influence of the tricalcium phosphate phase on the mechanical strength and biocompatibility of calcium phosphate bone cements

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INTRODUCTION: Polymethylmethacrylate (PMMA) bone cements are widely used in surgery to fix orthopaedic prostheses, such as joint replacements, in position and to repair bone defects for load-bearing applications. The other choice for bone cements is based on calcium phosphates [1]. Advantages of calcium phosphate (CaP) bone cements include good biocompatibility, bioactivity, bioresorption and the opportunity to tune the composition and properties. A setting temperature close to the body temperature prevents damage to the surrounding bone. The main disadvantage of CaP bone cements is the low mechanical strength [2] and this limits the application non-load-bearing sites for drug delivery systems and tissue engineering scaffolds [3]. The aim of this work is to investigate the solid phase composition for improve the mechanical properties and biocompatibility of CaP bone cements.

METHODS: Starting materials for the cement solid phase were α -tricalcium phosphate (α -TCP), nano-sized β -tricalcium phosphate (β -TCP) [4] or α -TCP/ β -TCP. A sodium phosphate buffer solution was used at a solid/liquid ratio of 1.75 g/ml to initiate the hardening reaction.

The phase composition and morphology of set CaP bone cements was obtained from X-ray powder diffraction, Fourier transform infrared spectroscopy and field emission scanning electron microscopy.

Compressive strength testing was determined on 7.5 mm diameter, 15 mm high cylinders at a cross-head speed of 1.00 mm/min⁻¹ using a screw-driven *Instron 4301*.

The biocompatibility of set CaP bone cements was investigated *in vitro* using the osteoblast-like cell line MC3T3-E1.

RESULTS: The low mechanical strength of nano-sized β -TCP as the sole ingredient suggests the need for α -TCP. This allows the combination of a reactive phase to provide a

cementing action together with the more stable β -TCP phase. Mixing two phases at different proportions showed that larger α -TCP content was desired to achieve cohesive mass with a sufficiently high mechanical strength. A 10–20 wt% β -TCP concentration provided a mechanical strength in the range of 11 to 18 MPa. This is equivalent to the compressive strength of trabecular bone (2–45 MPa) [5].

The results indicate that CaP bone cements contain both calcium deficient hydroxyapatite and β -TCP phases after setting

Cultured cells spread in a planar fashion, meaning that the cells were activated with strong adhesion on the surface. The osteoblast-like cell proliferation rate varied with the α -TCP/ β -TCP ratio and provided a maximum at a 90/10.

DISCUSSION & CONCLUSIONS: Nano-size β -TCP is not suitable by itself due to the low mechanical strength of the resulting bone cements, but it can be successfully used as a solid phase additive. The more stable β -TCP phase provides a better cell response, but the α -TCP phase is required to generate the required mechanical strength.

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From biological effects of gallium on bone cells to the design of gallium-doped calcium phosphate

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INTRODUCTION: The clinical context of this study is related to fractures caused by osteoporosis. Given that osteoporotic fractures mainly occur in specific bone sites such as proximal femur, their local prevention has recently been considered with interest. This strategy consists in locally reinforcing these bone sites by an implantable bioactive drug-combined biomaterial associating a calcium phosphate (CaP) bone substitute with an inhibitor of osteoclastic activity. Among the potential compounds, gallium (Ga) is a promising candidate due to its ability to substitute Ca in biological apatite. The chemical characteristics of Ga should presage the possibility of incorporating Ga into CaP biomaterials. Interestingly, Ga is clinically used for the treatment of hypercalcemia in the case of malignancy and Paget's disease, thereby suggesting a potent inhibitory effect of Ga on bone resorption. These chemical and biological properties have been extensively used to design a gallium-doped CaP [1, 2]. In the present study, we investigated cellular and molecular effects of Ga on bone cells.

METHODS: Using human and mouse models of osteoclastic differentiation, we performed real-time polymerase chain reaction analysis, western blot experiments, immunostaining assays and intracellular Ca^{2+} oscillation measurements.

RESULTS: Ga was found to dose-dependently inhibit the *in vitro* resorption activity of osteoclasts isolated from rabbit bone. Ga also reduced in a dose manner the formation of TRAP+ multinucleated cells and the differentiation of osteoclastic cell line (RAW 264.7). Ga treatment of human CD11b⁺ cells isolated from peripheral blood inhibited the expression of RANKL-induced early differentiation marker genes while the same treatment performed subsequently did not modify the expression of late differentiation marker genes. Focusing on the early stages of osteoclastic differentiation, and using mouse pre-osteoclasts, we observed that Ga

considerably disturbed both the initial induction as well as the auto amplification step of *Nfatc1* gene, which encodes a master transcription factor regulating osteoclastogenesis. Considering calcium signaling, we also observed upon Ga treatment a blockade of gadolinium-induced calcium entry through TRPV-5 calcium channels.

DISCUSSION & CONCLUSIONS: Ga exhibits a dose-dependent anti-osteoclastic effect by reducing *in vitro* osteoclastic resorption, differentiation and formation without negatively affecting osteoblasts. This inhibitory effects of Ga on osteoclastogenesis results from a significant reduction in the expression of NFATc1, a master regulator of RANK-induced osteoclastic differentiation. In addition, our results strongly suggest that the TRPV-5 calcium channel, which is located within the plasma membrane, is a target of Ga action on human osteoclast progenitor cells.

Considering biological effects of Ga, it seems justified to design a Ga-doped CaP.

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Mineralization of injectable inorganic-organic hybrid hyaluronic acid hydrogel

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INTRODUCTION: We have developed methods to induce the deposit of inorganic phases inside an organic hydrogel matrix. Hyaluronic acid (HA) modified with bisphosphonates (BP) via orthogonal chemistries was chosen as the backbone. The BP groups alone with calcium ions can act as nucleation points to help the growth of calcium phosphate particles [1].

METHODS: The gels formed by mixing two types of HA derivatives [2]: HA-aldehyde (HA-al) and HA-hydrazide-BP. The mineralization was done in a commercial available PBS containing calcium and magnesium ions. The gels were characterized by rheology, scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), et al.

RESULTS: We have confirmed the particle growth according to SEM. Calcium content assay also showed the increasing of calcium concentration with the mineralization time. The hybrid gel can be enzymatically degraded by hyaluronidase.

organic hydrogel matrix. This type of gel is injectable and might be used to improve bone regeneration. Further research will be to investigate the interactions of this gel with cells and in vivo study for the clinical applicability.

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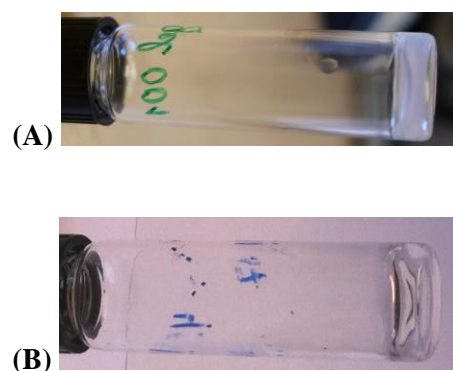


Fig. 1: Photographs of mineralized HA-BP hydrogel (A) and HA hydrogel without BP (B) as a control.

DISCUSSION & CONCLUSIONS: The use of orthogonal click modification opened a versatile way to prepare different kinds of HA derivatives including BP-linked HA. This polymer could attract cationic ions (calcium) and form the inorganic nanoparticles inside

The effect of injectable bone substitute on interface strength of a tibial prosthesis in a rabbit model

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INTRODUCTION: New antibiotic carriers for prevention and treatment of prosthetic infections are warranted and might on the same time add to the implant stability [1]. Injectable bone substitute composed of hydroxyapatite and calcium sulphate (Cerament®) was used as bone defect filler for the stabilization of tibial prosthetic components in an experimental rabbit model [2]. The aim of the study was to investigate and compare prosthetic fixation and tissue integration with and without the use of injectable bone substitute.

METHODS: Twelve skeletally mature (weight 4.0 to 4.5 kg) 6-month-old New Zealand rabbits were used for this experiment. A rabbit tibial prosthesis was designed for our study. The injectable bone substitute used was a biphasic material, consisting of 60% α -calcium sulfate hemihydrate and 40% hydroxyapatite, with a water-soluble radiocontrast agent iohexol (Cerament®, Bone Support AB, Lund, Sweden). Tibial prostheses, randomly with or without bone substitute, were implanted in left and right tibiae and harvested at 6 and 12 weeks. The stability of the prosthetic fixation was evaluated using an Instron/MTS machine with a pull-out test under displacement control at 2 mm min⁻¹. The force required for prosthetic failure to occur was recorded and statistically tested using paired Student's t-test. Histological as well as histomorphometric analysis was performed. The presence of a foreign body reaction, bone integration and ingrowth as well as the percentage of bone to prosthesis contact was evaluated using light microscopy.

RESULTS: No difference was found in the yield force between with and without Cerament®, respectively at 6 or 12 weeks. In general, the displacement force was higher in 12 weeks compared to 6 weeks (Table 1).

Table 1. Mean of maximum displacement force between prosthesis fixated with and without Cerament (Mean±SD).

Time	Without Cerament (N)	With Cerament (N)
6 weeks	131±58	152±58
12 weeks	173±59	222±32

Bone contact percentage was between 62 to 67% at 6 and 12 weeks with no significant difference between with and without Cerament® (Figure 1).

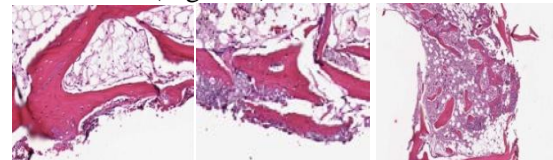


Fig. 1: Interface bone- prosthesis without Cerament (left) and, with Cerament (middle). Bone integration with Cerament in the distal part of the prosthesis (right).

DISCUSSION & CONCLUSIONS: The results of our study support that the use of Cerament® as a bone defect filler or gap filler around prosthetic joint implants result in a stable interface between the host bone and the implant providing stability. The use of Cerament® may serve as an alternative to autologous cancellous bone graft or allograft. Further a bone substitute may stabilize the prosthetic to bone interface in cementless prosthetic surgery and has the potential to act as an antibiotic bone conductive carrier.

REFERENCES: ¹Jensen J et al. Orthop Clin North Am. 2005;36:89-95. ²Wang JS et al. 2006, Abstract, American Orthopedic Research Society.

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Rheological properties of self-setting composite pastes for filling bone defects

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INTRODUCTION: Rheology is a key feature to describe the paste setting behaviour. The objective of this work was to study the influence of the hydroxyapatite modification and a liquid phase composition on the rheological behavior of the cement pastes, based on calcium phosphates (HA, MgHA and MgCHA) and calcium sulfate hemihydrate (CSH), during the early stages of setting. Distilled water and 1.0% chitosan solution in 0.3% acetic acid were used as liquid phases.

METHODS: Hydroxyapatite powders doped with Mg²⁺ or Mg²⁺, CO₃²⁻ ions were produced by wet chemical method. The cement powder phases were prepared by mixing modified hydroxyapatites and CSH powders in the weight ratio of 2:3. Viscosity of the investigated bone cements was studied as a function of time during the first period of setting. The experiment was performed using Anton Paar rheometer MCR 301 with parallel-disks sensor system which maintained constant stress and temperature. Time of samples preparation (mixing powders and liquids) and loading was 1.0 min. After that, recording of data points started immediately. The setting time of the cement pastes was determined using Gilmore Apparatus according to the ASTM C266-08 standard.

RESULTS: The liquid-to-powder (L/P) ratio necessary to obtain homogeneous and fluid pastes was 0.6 mL/g. The initial (I) and final (F) setting times of investigated cement type implant materials are presented in table 1 (Gilmore apparatus).

Powder phase	Liquid phase	Setting time	
		I[<i>min</i>]	F[<i>min</i>]
HA+CSH	Distilled water	13	24
	Chitosan solution	16	29
MgHA+CSH	Distilled water	18	41
	Chitosan solution	54	>60
MgCHA+CSH	Distilled water	12	33
	Chitosan solution	21	55

Figures 1-2 show the differences in the setting

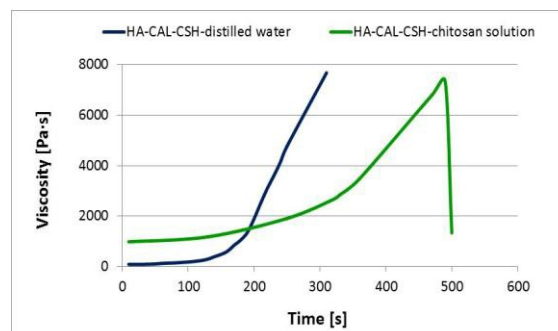


Fig.1 Viscosity vs. setting time of HA-CSH cements.

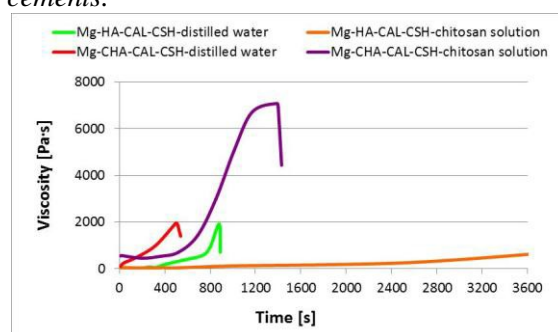


Fig.2 Viscosity vs. setting time of MgHA-CSH and MgCHA-CSH cements.

DISCUSSION & CONCLUSIONS:

Introduction of Mg²⁺ as well as Mg²⁺ and CO₃²⁻ ions into the hydroxyapatite structure caused growth in the initial and final setting time of the studied cements. Doping of hydroxyapatite with both, Mg²⁺ and CO₃²⁻ ions caused increase in the setting time in comparison to the cements based on the undoped hydroxyapatite, whereas for the materials composed of HA doped solely with magnesium ions decrease in the setting time was observed. The research conducted revealed a positive influence of the chitosan

cements. Lower and more stable increase of viscosity as compared to the materials in which

demonstrated. These features are crucial during the injection process, therefore the chitosan solution was assumed to be a liquid phase of choice in the case of the studied materials.

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