

Is kiva implant advantageous to balloon kyphoplasty in treating spinal osteolytic metastasis? a prospective randomized controlled study.

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INTRODUCTION: This prospective, parallel-group, controlled comparative randomized study compares the safety and efficacy of balloon kyphoplasty (BK) versus KIVA procedure for painful osteolytic vertebral body (VB) metastatic disease.

METHODS: From a total 44 patients, who suffered from intractable spinal pain because of vertebral fractures with evident osteolyses and suspected malignancy and who were initially enrolled in this prospective randomized study, 4 patients were excluded because the histologic study revealed no malignancy. Thus, this study examined 20 patients (71 ± 13 years) with 40 osteolytic vertebral body fractures, who received KIVA and PMMA and 20 patients (70 ± 11 years) with 40 osteolytic vertebral body fractures, who were reinforced with BK and PMMA. There were included 80 vertebral body osteolyses due to metastasis equally distributed in two groups. All procedures (80 vertebrae) were performed in 40 patients in a total of 40 sessions. Plain roentgenograms and CT-scans and MRI in some cases were used for vertebral parameters measurement, Tomita osteolysis location classification preoperatively, and for cement leakage detection shortly postoperatively. Anterior vertebral body height ratio (AVBHR), Posterior vertebral body height ratio (PVBHR), Middle vertebral body height ratio (MVBHR) vertebral wedge deformity (WD) were measured preoperatively to postoperatively in each osteolytic fracture level and comparisons were between the groups subsequently.

RESULTS: The documented osteolyses in both groups were graded as Tomita 1 to 7, average grade 5 and 4 in KIVA BK group respectively. KIVA was made from T₁₁ to S₂

level. BK was uniquely performed between T₇ and T₁₀, while KIVA below L₅ because of anatomy. Asymptomatic cement leakage occurred only in 4(10%) vertebrae (4 patients) in BK group: two anterior, one posterior to the spinal canal and one from the pedicle. No leakages observed to the disk or to the segmental vessels. All leakages occurred solely in cases of with Tomita grade 5 and 6 in BK group. No cement leakage occurred in the patients of the KIVA group. AVBHR and PVBHR improved similarly, although insignificantly in both groups but MVBHR improved marginally significantly ($P=0.07$) only in the KIVA group.

No significant changes occurred in WD in both groups. 87% of the patients noted immediately postoperatively marked or complete significant pain relief equally after BK and KIVA procedures. No patient was worse after treatment. There were no deaths related to the procedures.

DISCUSSION & CONCLUSIONS :

Percutaneous BK and KIVA provided immediately postoperatively significant spinal pain relief in a high percentage of cancer patients equally in both groups. The absence of cement leakage in the KIVA group and the low percentage of leakage in BK group without neurological impairment may reflect the safety of both augmentation techniques, the use of high-viscosity cement in BK and the safety of the KIVA PMMA containment technique and subsequent bone cement leakage control. Both KIVA and BK can safely stabilize vertebral osteolytic metastases grades 1-7 Tomita.

Thermoresponsive calcium phosphate cements

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INTRODUCTION: The goal of this work was to develop a thermoresponsive injectable calcium phosphate cement (CPC) for bone regeneration using alpha-tricalcium phosphate (α -TCP) as solid phase. The novelty of the work lies in the use of a poloxamer hydrogel as cement liquid phase. Poloxamers are amphiphilic triblock copolymers accepted by pharmacopeias for drug delivery applications. Poloxamer hydrogels present the advantage to be rheologically thermoresponsive increasing their viscosity with temperature. This property provided the cement with an excellent injectability close to room temperature and excellent cohesion at physiological conditions.

METHODS: α -TCP and beta-tricalcium phosphate (β -TCP) powders were obtained as described elsewhere¹. Solutions of 2.5 % Na_2HPO_4 with and without poloxamer F127 (20 wt%) were used as liquid phase. α -TCP and β -TCP were mixed with liquid phase at L/P ratio of 0.35 ml/g. Cement pastes were prepared at different initial temperatures (0, 7, 12, 15, 18 or 20 °C), and the extrusion behaviour was monitored through injection tests¹. Injection forces were determined from injection curves. Cohesion was assessed injecting the pastes in Ringer's solution (0.9 % NaCl) that was kept at different temperatures between 0 and 37 °C. Setting times were measured using Gilmore needles. The set cements were characterized after 7 days of setting at 37 °C in terms of the crystalline phases present (XRD), and the compressive strength.

RESULTS: Irrespective of the initial temperature of the paste (7 or 18 °C), shape retention of the α -TCP/poloxamer extruded strands increased with increasing temperature of the Ringer's solution, with total cohesion when Ringer's solution was at 37 °C (Fig. 1). In addition, α -TCP/poloxamer pastes were totally injectable, whereas in contrast the α -TCP paste without poloxamer presented phase separation, with a maximum plunger run of 55 %. Interestingly, the injection force decreased when decreasing the temperature of the paste, reaching a minimum value of ~50 N below 15 °C (Fig. 1). There were no significant

differences in injection force between α -TCP and β -TCP poloxamer pastes. This can be indicative that after mixing the addition of poloxamer introduced an induction period in the early stages of the setting reaction of α -TCP.

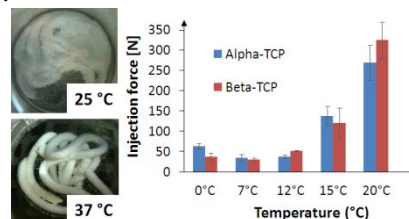


Fig. 1: Left: paste without cohesion in Ringer's solution at 25 °C and with cohesion in Ringer's solution at 37°C. Right: relation between the injection force and the temperature of the paste.

The α -TCP/poloxamer paste presented an initial setting time of 11.6 min and a final setting time of 33.7 min. α -TCP was completely transformed into calcium deficient hydroxyapatite after 7 days of setting. The compressive strength of the set cement was 28.4 ± 5.1 MPa.

DISCUSSION & CONCLUSIONS:

Poloxamer prevented phase separation, allowing complete cement injection, and conferred to the paste thermoresponsive properties. Thus, by controlling the temperature of the paste the rheological properties, and particularly the injection force can be modulated. Interestingly, α -TCP/poloxamer pastes showed complete cohesion in Ringer's solution at 37 °C irrespective to their initial temperature, without delaying their setting *in situ*. The set cement reached similar compressive strength than several commercial cements, which makes it an excellent injectable bone filler material.

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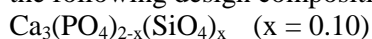
A solid state ^{31}P and ^{29}Si MAS NMR, ^{43}Ca DOR NMR, and GIPAW DFT study of α -tricalcium phosphate and Si-substituted α -tricalcium phosphate bioactive materials

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INTRODUCTION: Numerous calcium phosphate materials have generated significant interest as potential biomaterials due to their chemical and structural similarities to the apatite phases that constitute the mineral component of natural bone. Materials such as alpha-tricalcium phosphate (α -TCP: $\text{Ca}_3(\text{PO}_4)_2$) have been subject to increased attention recently since it demonstrates both resorbable and bioactive characteristics. The α -TCP ceramic is a metastable phase at room temperature; this means that the α -TCP structure needs to be ‘frozen’ in place from very high temperatures where it exists as a thermodynamically stable phase. Unlike the addition of other cations such as Mg^{2+} and Zn^{2+} , the addition of Si^{4+} helps to stabilise α -TCP to a lower temperature [1], thus making the α -TCP synthesis easier by alleviating the importance of the quenching temperature. This solid state NMR study investigates the structure of Si- α -TCP in relation to its α -TCP parent material.

METHODS: Samples of stoichiometric α -TCP and Si- α -TCP ($x = 0.10$) with no charge-balancing adjustments were prepared based on the following design composition:



thus maintaining the Ca/P+Si ratio the same as the initial parent Ca/P ratio [1,2,3].

^{29}Si and ^{31}P MAS, and ^{43}Ca DOR NMR measurements were performed at ambient temperatures (~ 297 K) on a Bruker AvanceIII 500 spectrometer ($B_0 = 11.7$ T) operating at ^{29}Si , ^{31}P and ^{43}Ca Larmor frequencies of 99.3, 202.4 and 33.6 MHz, respectively.

RESULTS: The ^{31}P MAS NMR data from high purity α -TCP and Si- α -TCP ($x = 0.10$) are compared in Figure 1, together with the corresponding XRD data characterising these systems. This comparison illustrates the marked differences that Si doping imparts on the TCP structure when comparing long-range and short-range length scales.

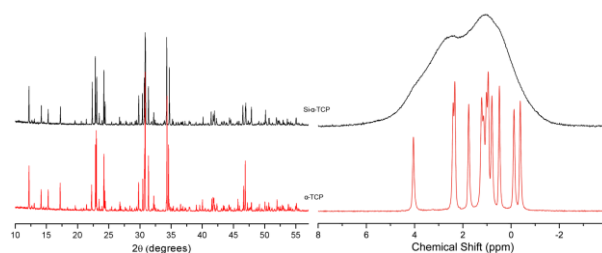


Fig. 1: XRD data and ^{31}P MAS NMR data for α -TCP ($\text{Ca}_3(\text{PO}_4)_2$) (bottom), and Si- α -TCP ($\text{Ca}_3(\text{PO}_4)_{2-x}(\text{SiO}_4)_x$ ($x = 0.10$)) (top).

DISCUSSION & CONCLUSIONS: The results of Figure 1 describe the extensive loss of short-range order (as described by the MAS NMR data) upon Si incorporation into the TCP structure, while the long-range order is largely preserved (as indicated by the XRD data). The ^{29}Si MAS NMR data from the Si- α -TCP system (not shown) clearly resolves contributions from both Q^0 (orthosilicate) and Q^1 (pyrosilicate) species that reside within the TCP framework, while the associated 2D ^{31}P - ^{29}Si HETCOR result (not shown) unambiguously places these Si species within the TCP structure and not as phase-separated surface moieties. Furthermore, the 2D ^{31}P - ^{29}Si HETCOR result shows that this Si speciation is not homogeneously distributed throughout the structure, but rather certain substitution positions are favoured. The ^{43}Ca DOR results (not shown) exhibit excellent resolution enhancement of the 18 Ca positions defining the α -TCP structure.

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Powder and liquid flow regimes during extrusion of calcium phosphate pastes.

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INTRODUCTION: Phase separation during injection of calcium phosphate cement (CPC) inhibits its application in percutaneous surgery. Phase separation of non-setting calcium phosphate paste (CPP) occurs when the liquid component flows more readily than granular flow of the powder [1]. The aim of this study was to determine the liquid flow regime and its effect on the deformable powder matrix during extrusion of CPP, identifying new approaches to improve CPC injectability and obtain homogeneous extrusion.

METHODS: CPP (6 mL) was loaded into a syringe and extruded at a constant rate to a maximum force of 100 N [1]. Variables investigated were liquid-to-powder ratio (LPR) (0.4, 0.425 and 0.45) and extrusion rate (5, 10 and 20 mm/min).

LPR of samples taken from the extrudate and paste remaining in the syringe barrel were determined. Consequently, flow regime of the liquid part of the CPP could be observed. To determine the deformation of the powder matrix, individual layers of powder were dyed a different colour and loaded into the syringe, creating a striped effect. Post injection, paste remaining in the syringe was removed and sliced transversely to the longitudinal axis, effectively revealing the cutaway section of the paste remaining in the barrel.

RESULTS AND DISCUSSION: In the barrel, the lowest LPR occurred at the plunger (Fig. 1a; A) and subsequently increased towards the exit (Fig. 1a; D). Due to inter particle and CPP-wall friction, the pressure exerted by the plunger is not transmitted uniformly throughout the paste, pressure being greatest at the plunger side. A rise in pressure increases liquid migration due to an increase in powder consolidation [2]. The LPR is highest directly above the exit point of the syringe barrel, due to the paste in this region being able to flow freely. However, the paste within corners (Fig. 1a; C) is constrained by syringe walls on the exit side, increasing pressure and liquid migration.

Table 1: Volume flow rate of liquid relative to powder during injection (times faster)

		Plunger rate (mm/min)		
		5	10	20
LPR	0.400	1.52	1.48	1.47
	0.425	1.59	1.51	1.46
	0.450	1.61	1.56	1.50

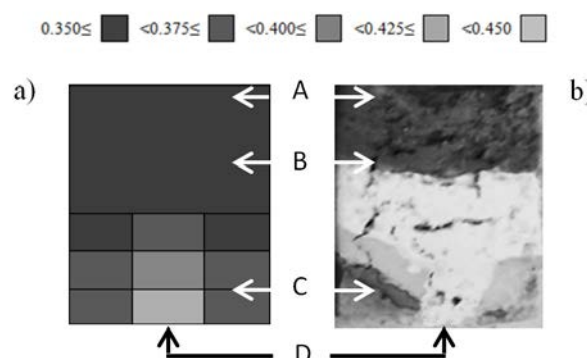


Figure 1: a) LPR distribution remaining in syringe barrel post injection. Key value represents LPR; b) Cutaway view of powder remaining in syringe barrel for an initial LPR of 0.425.

When powder flow is considered, line B in Fig. 1b remaining horizontal confirms plug flow occurs, indicating wall slip [3]. Plug flow does not continue at barrel exit. Preceding layers not fully extruded ('static zones') are evident (Fig 1b; C). Liquid migration towards the exit contributes to static zone formation, as regions of CPP with low LPR have an increased yield stress, decreasing flow relative to regions of high LPR. Similar trends were observed for all parameter combinations investigated; i.e. plug flow, static zones and similar LPR distributions.

Increasing plunger rate decreased phase separation (Table 1) due to less time for liquid migration through the powder matrix.

CONCLUSION: Liquid flow is dependent on pressure distribution within the syringe barrel. Consequently powder flow is dependent on flow of liquid; powder in regions of high LPR flowing more readily due to a decrease in yield stress. Results agree with previous findings [1] indicating methods to decrease yield stress and permeability, will reduce phase separation. Results also suggest increasing pressure distribution uniformity within the syringe can decrease phase separation.

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Separation phenomena occurring during the injection of β -tricalcium phosphate and glass beads aqueous pastes

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INTRODUCTION: One recent trend in the field of calcium phosphate cements (CPC) consists in introducing millimeter-sized particles in the pastes to create macroporosity or to release drugs, bioactive molecules and cells. However, the addition of large particles in CPC is likely to decrease the injectability of the pastes and cause separation phenomena between solid and liquid phases (= phase separation) and between small and large particles (= size separation). Therefore, the aim of this study was to investigate the effect of millimeter-sized spherical glass beads on the injectability and on separation phenomena of non-setting model pastes [1].

METHODS: The pastes consisted of a mixture of powder (P), glass beads (B) and liquid phase (L). P was β -Ca₃(PO₄)₂ (Sigma-Aldrich), L was either demineralized water or 0.5 wt % Na hyaluronate solution (Lifecore Biomedical) and different weights and sizes of B (Spheriglass®, Potters Europe) were introduced. After manual mixing of P+B+L, the paste was poured into a 1mL syringe (BD Luerlock, intern diameter: 4.5 mm, opening diameter: 1.9 mm). Injectability was measured (n ≥ 3) using a compression testing machine (rate = 0.4 mm.s⁻¹). Samples of the extruded paste were collected at 3 time points: in the first 20 mm of the piston displacement, between 20 and 40 mm, and after 40 mm (40-60 mm for a completely injectable paste). Measurements were stopped when the load required to extrude the paste from the syringe reached 250 N and the weight proportion of extruded paste was determined. Collected samples were submitted to successive treatments to determine weights of L, P and B in each portion of the extruded paste. L/(P+B), P/B ratios were then calculated. The size distribution of B within the different portions of paste was determined by image analysis. Statistical analysis was performed by ANOVA tests (p<0.01).

RESULTS: Adding from 0 to 40 wt.% of 156 μ m-sized beads into the pastes resulted in a significant injectability increase, whereas for

390 μ m-beads, the paste injectability remained constant until 36 wt.% and dropped then suddenly (Figure 1).

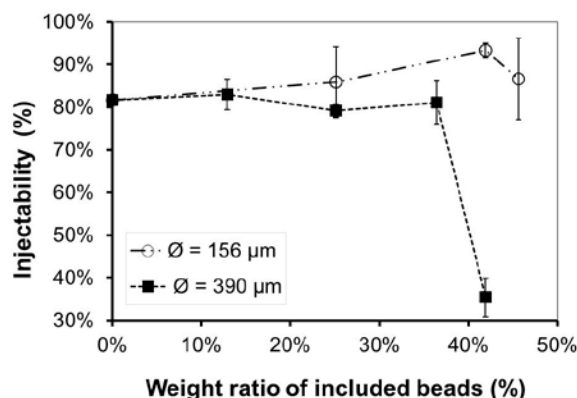


Fig. 1: Injectability of pastes vs. weight ratio of included beads (B diameters = 156 μ m or 390 μ m)

The L/(P+B) ratio of the extruded pastes did not vary throughout the injection but was significantly higher than the L/(P+B) ratio of the paste remaining in the syringe after extrusion, indicating the existence of phase separation. This effect was decreased with a more viscous L. The P/B ratios varied inversely: it increased along the plunger displacement, suggesting that beads were extruded whereas a dense cake of powder particles formed at the rear of the syringe. Finally, the particle size distribution of extruded beads shifted towards smaller sizes along the injection process, confirming that size separation occurred.

DISCUSSION & CONCLUSIONS: This study showed that phase and size separation occurring during the injection of model pastes were amplified by the addition of millimeter-sized beads. Except for the smallest tested bead size, a significant decrease of paste injectability was observed with increasing size and proportion of beads, as observed in previous studies for usual cement pastes when the size and the volume of the micrometer solid phase particles were increased. Moreover, two separation phenomena occurred simultaneously during the extrusion: both liquid and large particles (B) moved faster than small particles (P). This led to the formation of a dense powder cake at the rear of the syringe.

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Injectability of a new calcium aluminate cement for endodontic application (Endobinder)

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INTRODUCTION: Despite the clinical performance of Mineral Trioxide Aggregate (MTA), some of its negative features such as poor handling characteristics, long setting time and low flow capacity have led to the development of new root filling materials. “EndoBinder”(EB) (Binderware, São Carlos, SP, Brazil) is a calcium aluminate-based endodontic cement composed (%wt) of Al₂O₃ (≥68.0), CaO (≤ 31.0), SiO₂ (0.3-0.8), MgO (0.4-0.5) and Fe₂O₃ (<0.3). Assessment of its mechanical properties and microstructure¹, colour stability², antimicrobial activity and biocompatibility have been performed aiming to validate the new cement for dental application, however, its injectability via a needle has not been evaluated yet. The aim of this study was therefore to evaluate the injectability of Endobinder and to investigate the suitability of calcium nitrate addition to modify the extrusion and compressive strength (CS) of the cement.

METHODS: EB was manipulated in accordance with the manufacturer’s instructions, mixing the powder with distilled water at a powder-to-liquid ratio of 2.1g/ml. 5wt% of calcium nitrate was added to the modified cement. Pastes were hand mixed for 1 minute, then placed into a 5ml syringe and extruded through a 19 gauge stainless steel needle with inner diameter of 0.69mm. This needle size was selected due to the small inner diameter, indicating suitability for dental application. The cement was extruded using an universal mechanical testing machine at a cross-head speed of 30mm/min, which was continued until either the entire paste was extruded or a maximum force of 100N was reached. The weight percentage of paste extruded from the syringe was then determined.

RESULTS: Pure EB was not fully injectable, however, the addition of 5% Calcium nitrate improved the injectability without compromising CS (Figure 1).

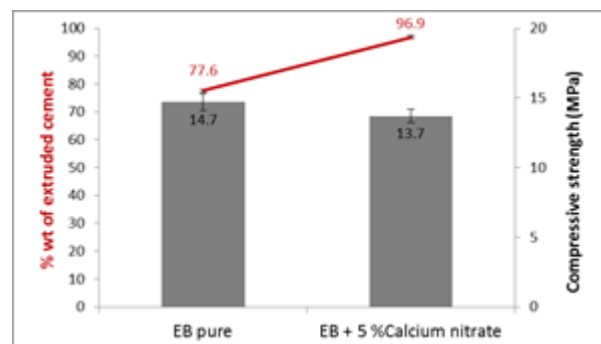


Fig. 1: Effect of Calcium nitrate on the extrusion and compressive strength of EB.

DISCUSSION & CONCLUSIONS: During the injection of pure EB, a liquid-phase separation could be observed, thus causing the limited injectability. Extrusion was not increased by lowering the PLR as this would not only have resulted in more porous cements, which are mechanically weaker, but also have promoted phase separation in the cement paste which would decrease injectability. Calcium nitrate acted as liquefying agent and no liquid-phase separation could be observed during extrusion, making the mixture fully injectable without decreasing CS. EB has a lower CS when compared with MTA. CS is assumed to be a less-critical factor for root-end filling materials³, as they do not bear direct mechanical load. However, as Endobinder is a multipurpose material that could also be used as a pulp capping or furcation repair material, the CS remains an important characteristic for the material to be considered and ideally enhanced.

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Long term clinical results from using SpineJack®

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INTRODUCTION: The aim of this study was to confirm the safety and clinical performance of the SpineJack® System in combination with Cohesion® Bone Cement for the treatment of vertebral compression fractures (VCF) due to trauma.

METHODS: This prospective international multicentric observational study was set up and conducted at fourteen centers across Europe including 103 patients, mean age at the time of the surgery was 61.6 years (SD 15.3) range from 18 to 88 years. 108 vertebral fractures were treated. In 81 patients, the fracture was only traumatic. In 22 patients, the trauma fracture was associated with osteoporosis. In this study 58.8% of the fractures are complex fractures: A2, A3 and B fractures. 27.9% of the fractures were classified as A.3.1, 4.8% as A.3.2 and 11.5% as A.3.3 fractures according to the AO Magerl classification.

The fracture age was a mean 9.2 (SD 7.8) days old at time of treatment.

Assessments of clinical parameters; Pain (VAS), functional capacity (Oswestry Global score, ODI), quality of life (EQ VAS) and analgesic intake were performed, together with radiological parameters, prior to surgery, after surgery and at 3 months.

RESULTS: Results were completed for 91 of the patients at 3 months follow up. The VAS score improved from 6.6 ± 2.6 at baseline to 1.4 ± 1.2 at 48 hours after the procedure and to 1.4 ± 1.7 at 3 months, representing a 79% ($p < 0.001$) overall improvement. The ODI, showing a decrease from 76.6 ± 19.4 % at baseline to 14.4 ± 17.4 % at 3 months postoperatively representing a 81% ($p < 0.001$) overall improvement. The results of the EQ-VAS are showing a significant increase of

Quality of Life from 50.4 ± 25.5 at baseline to 71.9 ± 24.4 at 3 months postoperatively. The decrease in pain allowed a dramatic and significant reduction in the intake of analgesics. 3 months after the surgery, 97.8% of patients were using no medication at all or only mild analgesics.

There were 7 new fractures, 4 of them adjacent, reported for 4 patients (3.9%). 3 patients (2.9%) presented fractures adjacent to the treated vertebra.

A statistically significant height restoration and endplates reconstruction with the SpineJack® were demonstrated immediately postoperative even with a high percentage of very complex fractures. In the group with severe fractures (A3), the mean value for highest point of restoration is 5.10 mm for the superior endplate and 2.52 mm for the inferior endplate. No implant related adverse events or serious adverse events were reported.

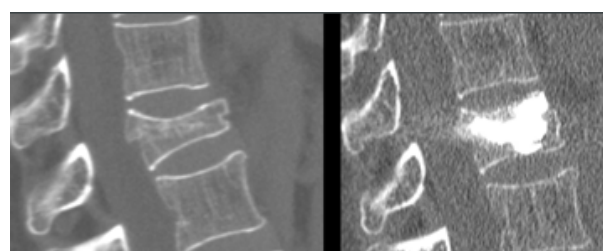


Fig. 1: 67 years old man, traumatic A.3.1 fracture in L1. Pre op and Post op CT scans.

DISCUSSION & CONCLUSIONS: Both the clinical and radiological results confirmed that SpineJack® procedure is a safe and efficient intervention for the treatment of traumatic VCF. The reported rate of adjacent fractures is 2.9% which can be considered very low compared to results presented in the literature.

Bone to go: Metal, ceramic or polymer?

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INTRODUCTION: Life expectancy nowadays, in most countries around the world, is around 80 years [1]. With a long life expectancy high health expectations come along. This defines needs to satisfy and markets opportunities are created. The bone substitutes market, in 2012, was around 222 million USD in America and around 200 million Euros in Europe [2, 3]. Depending on the area of application product choices to replace/substitute bone could be ample, *i.e.* metal, ceramic, polymer or a combination of these. This is the joint work of different actors which will allow finding the best alternative to the different needs.

Metals are able to load weight. Due to this characteristic most of the implants, *i.e.* orthopaedic, spine or dental, are made mainly of metal. Here, aspects of importance are the structure and surface. Structure can be obtained by different ways such as machining, the lost wax process, CAD/CAM, etc. Surface modification can also be reached, in order to increase the implant-host interaction, by several means, such as plasma spraying, electrochemically, radiofrequency plasma, etc [4-6]. Ceramics are more brittle than metals but offer other proven advantages, mainly on the reactivity aspect. These materials have been under research, in an intensive manner, since the 1980's and through the years their interest for minimal invasive applications has increased [7]. These materials offer the possibility to be used for cancer applications [8]. However, some aspects of their processing parameters need still to be clarified [9, 10]. Polymers have revolutionized our daily life and they will do the same on the bone substitute domain. In combination with the other two aforementioned materials, polymers can complement, or modify, the properties and characteristics of the final materials. So, they can be used as coatings for metallic implants or matrices/fillers for ceramic materials [11-12].

The way from bench to bed could be long and tortuous. In addition, the human body is a complex system; hence, basic and applied research should be performed in close interaction with veterinarians and clinicians in order to overcome obstacles together.

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Space maintenance and new bone formation with injectable and settable composite bone grafts in a canine mandibular ridge saddle defect model

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INTRODUCTION: Large mandibular defect reconstruction presents a continual challenge in oral and maxillofacial surgery. Growth factors, such as recombinant human bone morphogenetic protein-2 (rhBMP-2), incorporated into scaffolds stimulate new bone formation. Low-viscosity (LV) injectable polyurethane grafts have been reported to be effective carriers for rhBMP-2 and support new bone growth.¹ By reinforcing the scaffold with ceramic particles, its space maintenance properties can be enhanced. Mastergraft (MG) is a biocompatible, biphasic ceramic composed of 85% β -tricalcium phosphate and 15% hydroxyapatite that is similar in mineral content to natural bone.² 45S5 Bioglass (BG) is a bioactive, resorbable glass that has been used effectively in a variety of bone regeneration applications.³ In the present study, we investigated the ability of injectable LV-MG and LV-BG composites with two doses of rhBMP-2 to heal saddle defects in the canine mandible.

METHODS: LV was synthesized from a lysine triisocyanate (LTI)-polyethylene glycol (PEG) prepolymer, a polyester triol 450 (g/mol), and triethylene diamine catalyst. Treatment groups included the LV-45% MG or LV-45% BG with 100 μ g/mL (low dose, L) or 400 μ g/mL (high dose, H) rhBMP-2 (n=6/group). The lyophilized rhBMP-2 was hand-mixed into the PUR and injected into saddle defects in the canine mandible (4/animal) measuring 7-8 mm apicocoronal by 8-10 mm mesiodistal. The biocomposite was shaped through the creation of a pocket of soft tissue into which the composite could be injected (Fig 1A). The grafts expanded to ~50% porosity and hardened within 7-9 min, at which point the soft tissue was sutured over the defect sites. Animals were sacrificed at 16 weeks and new bone formation evaluated by radiography, μ CT, histology, and histomorphometry.

RESULTS: Images of mandibles treated with LV-BG or LV-MG augmented with 100 (L) or 400 (H) μ g/mL rhBMP-2 and explanted at 16 weeks are shown in Fig. 1. While all four grafts promoted bone healing, μ CT images reveal that LV-MG-H and LV-BG-H more effectively maintained the original height of the ridge. Quantitative measurements (Fig. 1B) showed a significant increase in ridge height at the high dose of

rhBMP-2, while differences between materials were not significant at either dose. Histology and histomorphometry are ongoing.

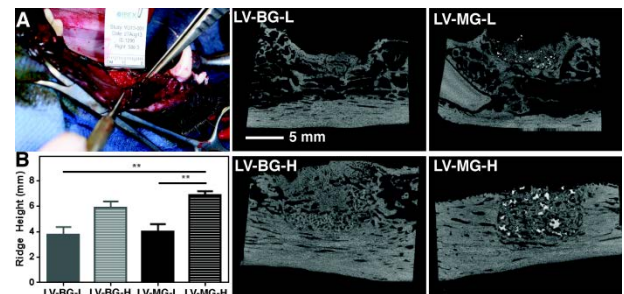


Fig. 1: (A) Photograph showing injection of LV grafts into saddle defects. (B) μ CT measurements indicate that the high (H, 400 μ g/mL) rhBMP-2 dose more effectively maintained ridge height than the low (L, 100 μ g/mL dose). μ CT images of saddle defects at 16 weeks reveal evidence of new bone formation.

DISCUSSION & CONCLUSIONS: Injectable, settable LV-MG and LV-BG composite bone grafts augmented with rhBMP-2 supported new bone formation and remodeling in canine mandibular ridge saddle defects. Maintenance of the height of the mandibular ridge increased with rhBMP-2 dose. Injectable LV grafts therefore provide the potential advantage of space maintenance and healing without the need for protective membranes or cages.⁴ LV grafts will be further evaluated in preclinical models of lateral ridge augmentation.

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Assessment of osteogenic potential of injectable human mesenchymal stem cells/coralline hydroxyapatite/calcium carbonate microtissues

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INTRODUCTION: Coralline hydroxyapatite/calcium carbonate (CHACC) is a biodegradable and osteoconductive bone graft material¹, which has demonstrated excellent clinical performance in our previous study². We hypothesise that CHACC can be used as a carrier for mesenchymal stem cells (MSCs) to form injectable microtissues which will have the potential to enhance osteogenesis. The aim of the study is to construct human mesenchymal stem cells on CHACC granules to form microtissue, and to assess their osteogenic differentiation in vitro.

METHODS: CHACC blocks were ground to granules using a mortar and pestle, and subsequently sieved to select the granules sized between 200 and 300 μm . Human MSCs isolated from bone marrow and umbilical cord matrix were used for comparison. Microtissues were formed via hanging-drops in Terasaki microplates, by adding 3,000 human MSCs with 3 CHACC granules/well (in 20 μl αMEM supplemented with 10% fetal bovine serum and 1% antibiotics), and incubated for 24 hours. The microtissues are then transferred to 100 mm petri dishes, and continuously cultured in either control or osteogenic differentiation medium (100 nM dexamethasone, 10 mM β -glycerol phosphate, and 100 μM 2-phosphate-ascorbic acid). Mesenchymal stem cell phenotypes were characterised using a BD flow cytometer for CD14, CD34, CD73, CD90, CD105, CD19 and CD45. Microtissues were harvested at days 1 (baseline), 7, 14 and 21 days and imaged using bright field microscopy and scanning electron microscopy (SEM). The samples were fixed in 10% Formalin and fluorescently stained for alkaline phosphatase. At day 21, immunocytochemistry of osteocalcin, osteopontin, RUNX2 and type I collagen were performed and total RNA from the microtissues were extracted for qPCR analysis of RUNX2, type I collagen, ALP, Osteopontin and Osteocalcin expression.

RESULTS: Human MSCs enveloped the surface of CHACC granules to form microtissue at day 1 after seeding. Cells increased in number continuously and bridged nearby microtissues at day 7. At day 21 all CHACC granules were integrated by MSCs and their extracellular matrixes (Figure 1). Human MSCs from bone marrow and umbilical cord matrix both showed osteogenic differentiation, assessed by immunohistochemistry and qPCR of osteogenic markers of RUNX2, Collagen type I, osteocalcin and osteopontin. However, these markers were more pronounced in human MSCs from bone marrow than those from umbilical cord matrix.

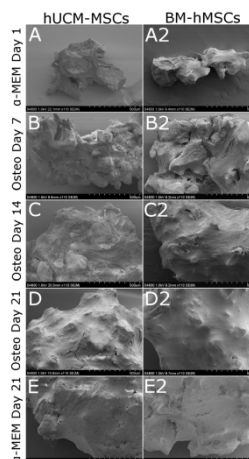


Figure 1: SEM images of hUCM-MSCs (A-E) and BM-hMSCs (A2-E2) grown on CHACC. Control $\alpha\text{-MEM}$ images at day 1 and 21 of hUCM-MSCs (A and E, respectively) and BM-hMSCs (A2 and E2, respectively). Human UCM-MSCs osteogenically induced at day 7, 14, and 21 (B, C, and D, respectively) and BM-hMSCs (B2, C2, and D2, respectively). Scale bars 500 μm .

DISCUSSION & CONCLUSIONS: CHACC granules can be a suitable carrier for human MSC proliferation and differentiation in vitro, for the purpose of injectable delivery. However, as expected, MSCs from bone marrow have superior osteogenic differentiation abilities to those from umbilical cord matrix.

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FTIR-Attenuated Total Reflectance: a new tool in the assessment of IVD aging and degeneration.

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INTRODUCTION: Aging and degeneration cause significant alterations in the biochemical composition of the IVD tissue with respect to these components¹. In this context, the objective of this study was to demonstrate the efficacy of Fourier transform infrared-Attenuated Total Reflectance (FTIR-ATR) as a reliable technique to detect major components of the IVD extracellular matrix (ECM) and discerning subsequent changes to IVD tissue in two complementary models of IVD degeneration disease (DDD).

METHODS: Age-related DDD model was performed using New Zealand White rabbits of increasing ages (1, 6 and 24 months). In addition, enzymatic treatment with hyaluronidase (1mg/20 μ L) was used to induce an experimental model of DDD in 1 year-old rabbits². Longitudinal follow-up in experimentally-induced animal model was performed at 7, 30 and 60 days. MRI (T2 sequence) was used to assess the Pfirrmann's grading and to stratify IVD in several groups with increasing severity of DDD: grade I, II and III and IV. In-situ analysis with FTIR-ATR was conducted to determine the chemical composition of IVD. Nucleus pulposus (NP) and Annulus fibrosus (AF) of each IVD was examined by FTIR-ATR and spectrum analyzed.

RESULTS: In the age-related model, three groups have been defined according to Pfirrmann's grading (grade I, II and III). Interestingly, spectra in NP (Fig. 1) and AF (data not shown) showed reproducible modifications notably in the spectral regions of 1340, 1550, 2850, 2960 cm^{-1} . In experimentally-induced DDD model, spectra of control IVD (grade II with no enzymatic treatment) show at day 7 a FTIR-ATR spectrum quite comparable to the spontaneous IVD spectrum of grade II (data not shown). Enzymatic treatment induces rapidly a deterioration of the Pfirrmann's grading from grade III to IV-V. Consistently with this Pfirrmann's grading increase, a drastic modification of FTIR-ATR spectrum is observed at day 7 and 60 (Fig. 2).

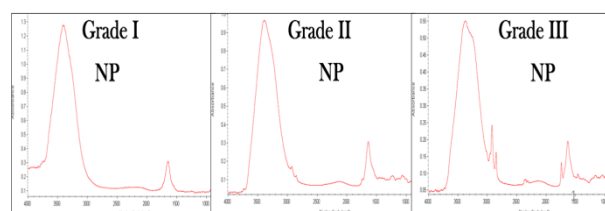


Fig. 1: FTIR-ATR spectrum of NP in spontaneous model according to IVD Pfirrmann's grading.

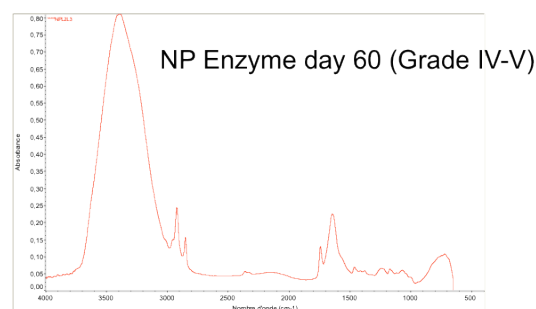


Fig. 2: FTIR-ATR spectrum of NP in experimentally-induced animal model according to IVD Pfirrmann's grading.

DISCUSSION & CONCLUSIONS: These results illustrated that FTIR-ATR can be used as an easy and fast ex vivo analysis of molecular constituents of IVD. The molecular information obtained may be relevant for understanding the pathogenesis of IVD degeneration. Identical modifications between grade III in aging IVD and in grade IV-V after enzymatic treatment confirm that some spectrum regions could be specific of macromolecular complex (proteoglycans and collagens) alteration in the ECM of IVD.

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High frequency impedance measurement for the relevant determination of setting times of apatitic cement

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INTRODUCTION: The discovery of calcium phosphate cements (CPC) constitutes a significant advance in the field of biomaterials for bone reconstruction, especially for injectable compositions which allow implantation using minimally invasive surgical techniques. In this context, one current trend is to formulate new CPC to obtain biocompatible and resorbable materials showing improved mechanical properties, allowing the delivery of drugs and together with a setting reaction proceeding in a suitable time. Therefore, determining the initial and final setting times of injectable bone cements is of great importance to define the time window for which the cement can be handled. For that purpose, two standardized methods are classically used: the Gillmore[1] or Vicat[2] needle tests. These methods are based on the concept of 'visible indentation' which is operator-dependent and can result in poor reproducibility from one research group to another, and are unable to provide information about the progress of the chemical reaction controlling the setting process. Monitoring chemical characteristics and physical properties of the cement as a function of time involves sophisticated characterization techniques with destructive conditions of preparation on collected samples.

RESULTS, DISCUSSION & CONCLUSION: We demonstrate, here, that the high frequency impedance (0.4-100MHz frequency range) allows non-destructive and *in situ* dynamic monitoring of setting reactions of cements dedicated to orthopaedic applications[3,4] (Fig.1). The present approach enables to identify in a non-invasive way through the variation of dielectric permittivity, ϵ' , and dielectric losses, ϵ'' : (i) a first weakly evolving step (with in some cases the presence of an induction period) for which the paste is not consolidated (ii) a second step showing sharp variations of the ϵ' and ϵ'' curves as a result of the conversion of α -TCP into CDA on the particles surface and (iii) a final stage for

which the dielectric permittivity and dielectric losses start to stabilize when the chemical transformation of α -TCP progresses towards the inner part of the particles. Unambiguously, the high impedance frequency is also likely to characterize the effect of additives (i.e, antiosteoporotic bisphosphonate drugs such as Alendronate) on the hydration process. Interestingly, the variation of both dielectric parameters not only gave clear evidence of the retarding effect of alendronate, but was also sensitive to the different mechanisms of hydration taking place depending on the way the BP was introduced in the cement formulation. It was thus observed, that the retarding effect was minimized when the BP was chemisorbed onto CDA, in agreement with ³¹P MAS RMN experiments.

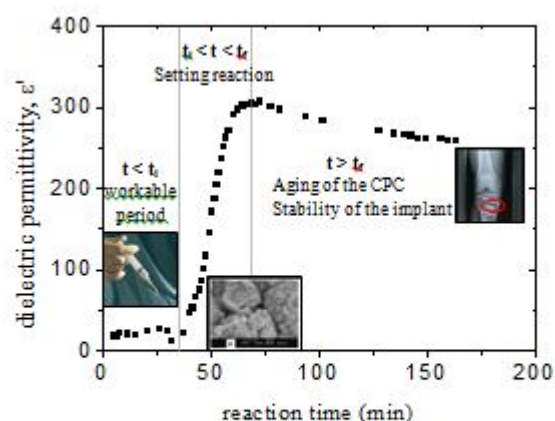


Fig. 1: Variation of dielectric permittivity, ϵ' versus reaction time for the undoped CPC.

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Calcium phosphate foams: Tunable, ready-to-use bone grafts

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INTRODUCTION: Calcium phosphate foams (CPFs) are synthetic bone graft substitutes presenting attractive properties such as an additional interconnected macroporosity compared to Calcium Phosphate Cements. This work explores the potential of different formulations of CPFs to tune their properties allowing their storage and readiness-to-use.

METHODS: The powder phase was a mixture of α -TCP containing 2%wt of precipitated hydroxyapatite and Pluronic F-127 (0, 10 or 15%wt). The liquid phase was a solution of 1%wt Tween80 in water. Calcium phosphate foams were prepared using a L/P ratio of 0.55mL/g, injected into moulds (6mm \varnothing , 12mm) and characterised after 7 days of setting. The compressive strength (CS) was evaluated. The total porosity and pore entrance size distribution (PESD) were evaluated by Mercury Intrusion Porosimetry (MIP). Right after preparation, some samples were frozen (-80°C). To unfreeze, a milk heater (Medela) was used, and the material was subsequently injected into moulds for setting.

RESULTS: CPFs clearly showed a macroporous structure (Fig.1) in all formulations.

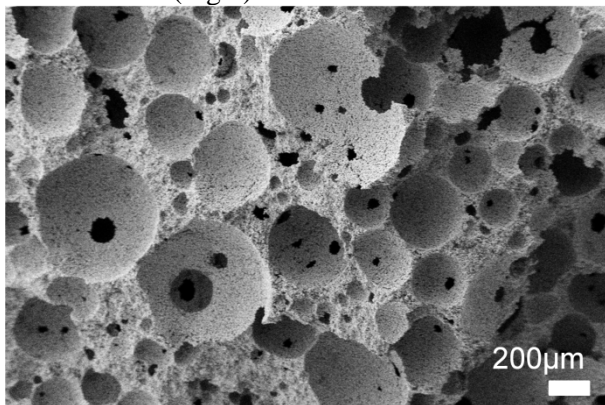


Fig.1: SEM Image of a pristine CPF showing its interconnected macroporosity.

The addition of Pluronic in the matrix led to a decrease in the pore interconnection diameter as recorded by MIP (Fig.2), and an increase in the CS while the total porosity was maintained (Fig.3).

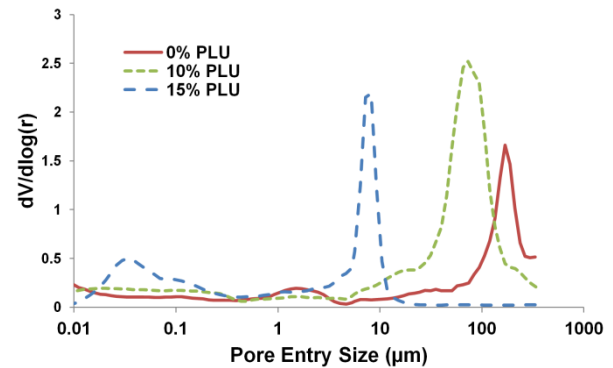


Fig.2: PESD of CPFs containing variable amounts of Pluronic.

Moreover, the freezing-unfreezing process did not significantly affect the macroporosity and total porosity of the CPFs as shown in Fig.3.

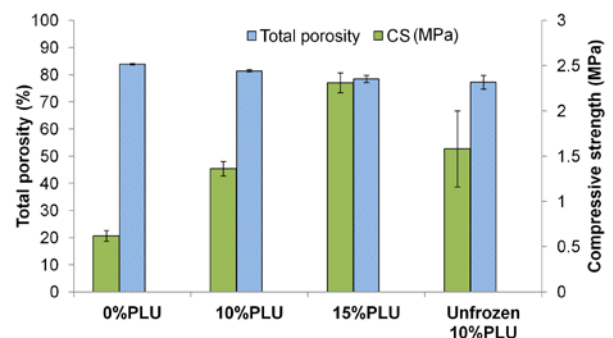


Fig.3: Total porosity and compressive strength of Pluronic containing CPFs, and unfrozen CPF.

DISCUSSION & CONCLUSIONS: Pluronic was used to tune the interconnection size of CPFs from 120 μ m down to 8 μ m. While the total porosity was not affected, the CS increased due to the decreasing pore size, due to the higher concentration of surfactant leading to the formation of smaller bubbles while foaming. The CPF containing 10% of Pluronic showed interconnections between pores around 80 μ m. It was shown that it is possible to freeze, store and deliver ready-to-use CPFs without altering their initial properties.

ACKNOWLEDGEMENTS:

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Biological and reconstructive properties of gallium-doped calcium phosphate bone substitutes

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INTRODUCTION: Calcium phosphate (CaP) biomaterials are commonly used in bone reconstructive surgery, and if combined with drugs, can serve as an active vector for local drug delivery such as the semimetallic element gallium (Ga). Ga has been used in treatment of several disorders associated with accelerated bone mineral resorption [1], and it was shown to inhibit the differentiation of osteoclasts, the bone-resorbing cells [2]. It was also demonstrated that Ga can be chemically associated with CaP following an ionic substitution of calcium sites [3]. Therefore, we embarked on investigating biological properties of novel gallium-doped CaP biomaterials using an *in vitro* co-culture model of osteoclastic differentiation and an *in vivo* rat model of defect healing.

METHODS: *In vitro*. CaP cement (CPC) and CPC-Ga pellets were prepared (following user manual for Graftys[®] Quickset). Human primary osteoblasts or human primary monocytes were seeded separately or together on cement pellets. At day 14, cells were TRAP-stained for qualitative observations. Alternatively, mRNA were prepared for quantitative RT-PCR. *In vivo*. A cylindrical critical-sized defect was created bilaterally at the distal femoral metaphysis of male Lewis rats. The osseous cavity was filled with micro-particles (80-200µm) of CPC+/- Ga or calcium-deficient apatite CDA +/- Ga. Animals were sacrificed 2 or 8 weeks after implantation and bone specimens were retrieved. 2D analysis from SEM images and 3D analysis from micro-CT images (Skyscan 1172) were conducted to determine histomorphological parameters. In addition, histological studies quantified the surface of newly formed tissue and micro particles.

RESULTS: *In vitro*. TRAP staining revealed mature osteoclasts on both CPC and CPC-Ga pellets. Furthermore, a decrease in osteoclasts number was observed on the surface of CPC-Ga compared to CPC pellets. *In vivo*. For all tested scaffolds, histological staining revealed a good osseointegration of implants into the surrounding host tissue accompanied by

successful bone ingrowth and bone marrow reconstruction (Fig.1).

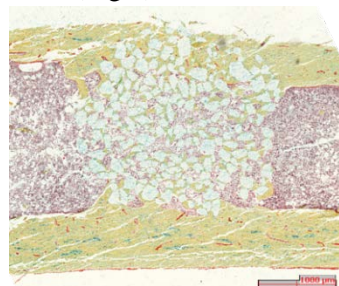


Fig. 1: Histological section stained by MOVAT (condition CPC at 2 months)

Preliminary results showed that defects implanted with CDA-Ga had a higher percentage of filling compared to control CDA, as observed by quantitative micro-CT analysis for samples at 2 months.

DISCUSSION & CONCLUSIONS: Here we demonstrate that CPC and CPC-Ga provide biocompatible and non-cytotoxic substrates for bone cells survival, thus allowing for their functional dialogue and osteoclastic differentiation. However, osteoclasts number is reduced on CPC-Ga pellets, potentially suggesting an inhibition of osteoclastic differentiation, which is yet to be confirmed by quantitative RT-PCR. In quantitative micro-CT analysis, *in vivo* model suggests an increase of the defect filling at 2 months in the case of CDA-Ga compared to control, and quantitative histological analysis is currently in progress to refine and further enforce these results.

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DEVELOPMENT OF A NOVEL INJECTABLE STRONTIUM CONTAINING BONE SUBSTITUTE

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INTRODUCTION: The aim of this study was to investigate the effect on cement properties and bone cells of progressive Sr-substitution for Ca in bioactive glasses used as precursors for novel calcium phosphate cements (CPCs). This novel route of cement production has been patented¹ and properties and pro-osteogenic effects have already been assessed in-vitro and in-vivo². They possess excellent osteoconductive properties and optimum resorption rate. The addition of Sr is hypothesised to improve their properties and to enhance performance in-vivo when this material is used as bone graft. Sr has been shown to stimulate in-vitro and in-vivo replication of pre-osteoblasts and to inhibit osteoclasts differentiation³. As a consequence faster rates of new bone formation and enhanced osteoconduction are the advantages of using Sr containing bone substitutes. Sr has been shown to increase radiopacity and its substitution for Ca in bioactive glasses is known to expand the glass network resulting in faster glass dissolution and improved bioactivity.

METHODS: Glasses were produced by progressively substituting Sr for Ca on a molar basis. Cements were prepared by mixing the glass powder with $\text{Ca}(\text{H}_2\text{PO}_4)_2$ powder and using 2.5% solution of Na_2HPO_4 solution (L/P 0.70) to start the setting reaction. Setting times and compressive strength were measured after 1h, 1d, 7d and 28d immersion in TRIS buffer solution on cylindrical samples. X-Ray Diffraction (XRD) and Radiopacity were assessed. Cell culture was performed using mouse and human osteosarcoma preosteoblasts and the samples observed by Scanning Electron Microscopy (SEM).

Table 1. Glass Compositions for Cements (mole %)

Glass	SiO ₂	P ₂ O ₅	CaO	SrO	Na ₂ O
ADSr0	42.00	4.00	39.00	0.00	15.00
ADSr5	42.00	4.00	37.05	1.95	15.00
ADSr10	42.00	4.00	35.10	3.90	15.00
ADSr25	42.00	4.00	29.25	9.75	15.00
ADSr50	42.00	4.00	19.50	19.5	15.00
ADSr75	42.00	4.00	9.75	29.25	15.00
ADSr100	42.00	4.00	0.00	39.00	15.00

RESULTS: Setting time increased proportionally with Sr-substitution in the glass up to 25%, whereas for higher substitutions it decreased. Compressive strength showed the same trend with a maximum medium value of 12.5 MPa for 25%Sr composition after 1d. XRD showed that the presence of Sr influenced the crystal

phases forming during the time and crystallites size. OCP ($\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$) was the main phase present after 1h and 1d. After 28d OCP was completely transformed to Sr-containing hydroxyapatite ($\text{Sr}_x\text{Ca}_{(10-x)}(\text{PO}_4)_6(\text{OH})_2$, SrHA). Radiopacity increased proportionally to Sr content. In general, fewer cells grew on the surface of 0% Sr compared to that of 25% Sr.

DISCUSSION & CONCLUSIONS: A novel method to develop a bone substitute forming *in vitro* SrHA as a final product by using a bioactive glass as a precursor was shown. SrHA appeared after the formation of OCP, which is believed to be a precursor in HA formation. Diffraction lines for HA showed a shift towards lower 2θ values with increasing in Sr substitution in the glass, meaning that the Sr was incorporated in the lattice of HA with evident effects on the unit cell and crystallite sizes. A correlation between the phases formed, setting time and compressive strength was witnessed. The compressive strength obtained in vitro matched the strength value of trabecular bone. Sr-addition enhanced cellular attachment and proliferation and increased radiopacity. Cements highly radiopaque are more easily tracked for their resorption and replacement by new bone. These novel bioactive glass cements are promising materials for dental and orthopaedic applications, resulting in a new more effective and biocompatible bone substitute. Further in- vivo characterizations are being conducted.

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Fabrication and characterization of bone cement-reactive poly(vinyl alcohol) membrane for balloon kyphoplasty

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INTRODUCTION: Balloon kyphoplasty is a typical operation technique in which the vertebral body is inflated with a balloon, after which poly (methyl methacrylate) (PMMA)-based bone cement is injected into the collapsed space. This operation technique is effective to reduce pain, however, its major disadvantage is leakage of the cement into the closed collapsed space of the vertebral body. Therefore, the development of a device that regenerates the vertebral body without cement leakage is required in clinical field. In the present study, we employed a poly(vinyl alcohol) (PVA) membrane as a starting material for implantable balloon kyphoplasty. PVA membrane has excellent mechanical properties derived from their intra- and intermolecular hydrogen bonding. Also, it is known that bone-like hydroxyapatite deposits on/in PVA hydrogels, meaning that implanted PVA membranes can be immobilized inside of vertebral body via calcified products such as hydroxyapatite when implanted in the body. Using PVA membrane, we introduced vinyl groups on the surface of the PVA membrane so that the bone cement can covalently react with the hydrogel membrane. Then, we evaluated the effect of this vinyl group modification of the PVA membrane on the bonding strength between the membrane and bone cement [1].

METHODS: Introduction of vinyl groups on PVA membrane was carried out as follows; Dried PVA membranes were immersed for 24 h at room temperature in glycidyl methacrylate (GMA) glycidyl aqueous solution adjusted to pH 1.5 using hydrochloric acid. After the reaction, the membrane was washed with excess acetone and water, and dried at room temperature. The resulting GMA-modified PVA (GMA-PVA) membranes were characterized using water contact angle measurement, fourier transform infrared (FT-IR) spectra, X-ray photoelectron

spectroscopy (XPS) and bonding strength measurement with PMMA bone cement.

RESULTS: The water contact angle of the PVA membrane was $45.6^\circ \pm 3.5^\circ$, which increased to $69.7^\circ \pm 3.5^\circ$ upon GMA modification, confirming that the hydrophobic GMA was actually introduced onto the PVA membrane. From FT-IR analysis, the peaks at 1700 cm^{-1} and $1200\text{-}1040\text{ cm}^{-1}$ were assigned to the C=O groups and C-O-C groups of GMA, respectively, suggesting that the hydroxyl groups in PVA were replaced with GMA groups. From XPS analysis, C1s narrow XPS spectra of PVA before and after GMA modification. Only the C-O peak was found on the original PVA membrane, while, C=O peaks appeared after modification with GMA, suggesting that the hydroxyl groups of PVA were partially converted to GMA groups. The prepared membrane adhered to PMMA-based bone cements within 10 min, which is the time required for PMMA polymerization in the bone cement. The bonding strength between the GMA-PVA membrane and the PMMA-based bone cement was higher than that for the original PVA membrane, likely because vinyl bonds form between the surface of the GMA-PVA membrane and the bone cement. Since GMA-PVA membrane adhered firmly to the PMMA-based bone cement, the membrane was able to completely cover the PMMA-based bone cement.

CONCLUSIONS: We developed PVA membrane that is reactive with PMMA-based bone cement by introducing GMA onto a thermally treated PVA membrane. The bonding strength between the PMMA-based bone cement and the GMA-PVA membrane was 2-fold higher compared with the original PVA membrane. The GMA-PVA membrane is therefore a useful material for implantable balloon kyphoplasty.

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Association of a novel peptide inhibitor targeting RANK and a crosslinked hydrogel as anti-osteoporotic drug delivery system

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INTRODUCTION: Due to the aging population of the industrial world, the development of novel therapies against osteolytic diseases is a subject of growing interest. Bone remodeling is characterized by a balance between osteoblasts expressing receptor activator of nuclear factor- κ B ligand (RANKL) and RANK-expressing osteoclasts [1]. One of the strategies consists in blocking the RANK/RANKL interaction with a peptide able to specifically bind to the receptor RANK. To avoid secondary effects of systemic therapies, drug delivery systems are of key importance.

Thus the aim of this study was first to develop a novel structure-based approach to generate peptides targeting RANK, then to build a tridimensional matrix able to encapsulate and slowly release such molecules.

METHODS: To identify inhibitors of RANK, a peptide library was generated *in silico*. After refinement process, 40 peptides were purchased from GeneCust EUROPE. The biological activity of each candidate was tested *in vitro* in an assay of osteoclast differentiation (human CD14⁺ and murine CD11b⁺ monocytes). The effect of the most promising peptide on RANK signaling was evaluated by qPCR and Western blot analysis. Finally *in vivo* experiments on ovariectomized mice were performed with daily subcutaneous injection of the peptide for 5 weeks and the activity of the peptide on bone remodelling was evaluated by microtomography and histology.

Chemically crosslinked hydrogels made of hydroxy propyl methyl cellulose (HPMC) were obtained by radiation. The swelling and the crosslinking ratio were measured in phosphate buffer saline solution.

The encapsulation of the peptide was performed by solubilisation in the polymer solution and crosslinking by irradiation. The kinetic release was studied in a physiological solution and monitored over 6 weeks by high performance liquid chromatography and (indirectly) via measuring the viability of mouse osteosarcoma (POS-1) cells.

RESULTS: A library of short peptides (22.5×10^6) was screened *in silico* according to the structure of RANK in complex with RANKL and refined based on their solubility in biologically compatible solvents (25,000) and their binding affinities with the receptor (~1,000). 50 peptides were tested *in vitro*; three molecules PepA3, Pep5_17K and Pep8 showed a high activity *in vitro* on human and murine osteoclastogenesis. Of these, Pep8 also had the highest solubility in aqueous solution, making it a promising candidate for further *in vivo* testing.

The inhibitory effect of Pep8 was confirmed by microtomography and histological analysis on ovariectomy-induced bone loss.

Concerning the HPMC hydrogels, the swelling and the crosslinking ratio were controlled by the radiation dose and the polymer concentration.

Kinetic release of the peptide was observed up to 4 weeks whatever the hydrogel formulation.

DISCUSSION & CONCLUSIONS: The *in silico* conception of peptide able to inhibit the RANK-RANKL interaction has led to novel and innovative molecules. The *in vitro* and *in vivo* experiments have validated the specificity of one peptide.[3] A patent has been issued on the amino acid sequence showing a strong inhibitory effect on osteoclastogenesis.[4]

The process of hydrogel formation showed that the network can be adapted depending on the size of the molecule to be released, leading to a sterile, ready-to-use drug delivery system. This technology was also patented.[5]

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Impact evaluation of an osteosynthesis device dedicated to prevent hip fracture

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INTRODUCTION: The second fracture of the upper part of the femur is associated to a dramatic increase of the mortality rate (from 20 to 50% depending of the studies) [1,2]. Therefore, it is clearly important to prevent this second fracture. The purpose of this study is to evaluate the impact of a new prevention dedicated osteosynthesis implant (PDOI) on patient in terms of safety and effectiveness.

METHODS: The study was performed in an on-going, prospective series of 15 PDOIs. To date, 3 patients were implanted. The PDOI was implanted into the contralateral hip during the same surgery time of fractured hip gamma nail implantation. Mean follow-up was 3 months. Clinical evaluation included the Oxford hip score, the WOMAC scores. Plantar pressure measurements were evaluated at 3 weeks and 3 months after the surgery using a Win-Pod (Medicapteurs).

RESULTS: Mean Age and BMI of patients were 83 ± 3 years and 25 ± 9 kg/m² respectively. Mean duration of surgery was 43 minutes (range 35 to 58). Cement quantities were similar over the 3 patients (6-7 cc). At 3 weeks, comparison between the two legs' plantar pressures revealed no differences (50%-50%). Experiences of pain were comparable between the two legs (0.7 and 1 for the Gamma Nail and the PDOI respectively). At 3 months, Womac scores for pain and functionality were 6 and 36 respectively and an OHS score of 25. Experiences of pain were similar between the two legs (3 and 4 for the Gamma Nail and the PDOI respectively). Concerning the plantar pressures, results obtained were in favor of the PDOI compared to the Gamma nail (53% vs. 47%). No osteolysis or implant loosening was observed at the different follow-ups.

DISCUSSION & CONCLUSIONS: At 3 months, patients have maintained good physical health without any inconveniences, in the contralateral hip, caused by the implantation of the PDOI. Furthermore, patients tend to more bear their weight on the leg with the PDOI. These first results are very encouraging and suggest that PDOI did

not cause additional troubles or pains to the patient.



Fig. 1: Per-operative radiography of the patient 1 just after implantation of the PDOI.

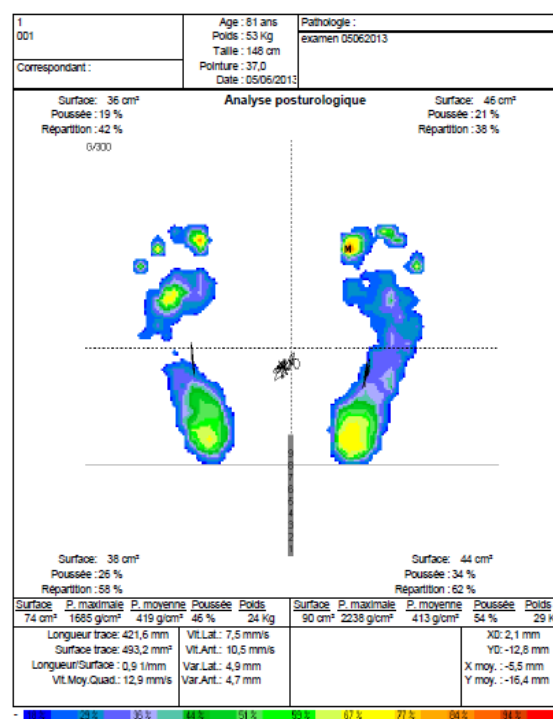


Fig. 2: Plantar pressure results of patient 1 at 3 weeks after the surgery. Similar pressure obtained in both left and right sides.

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Bone Augmentation Solutions Syndicate (BASS): A collaborative development of bone augmentation solutions for proximal humeral and hip fractures,

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INTRODUCTION: in proximal fractures of the humerus and the femur in elderly osteoporotic patients complication rates due to failure of screw/bone fixation may be as high as 40% and 8% respectively^{1,2}. Injectable CaP cement augmentation of fixation screws offers potential clinical benefits of reduced displacement and pain postoperatively³. However injectable cement makers often focus less on development of indication specific delivery devices.

To address unmet needs several smaller companies (see author list above) have formed a collaborative group – Bone Augmentation Solutions Syndicate (BASS) to ensure all aspects of clinical user needs are addressed in injectable biomaterials treatments.

METHODS: The six groups involved in this initiative contributed as follows (in order of author above): *clinical applications knowhow, relevant clinician experience, an implant designed for cement delivery, a novel CaP pre-mix cement, project management infrastructure, and mixing and delivery technologies.* The starting point was defining clinical user needs in key specific indications. This led the group to develop: specific flexible couplings, disposable and non-disposable cement delivery/pressurisation devices and specialty cannula designs. The new solutions were benchmarked by surgeons against current standard solutions

RESULTS: In the proximal humeral application, up to 6 screws may be augmented with CaP at one session requiring start/stop cement injection over a 10 minute period in a limited operative field. The BASS device package was assessed by clinicians with experience of current clinical practice⁴. The package for proximal humerus comprised cement injection screw implant, a flexible implant to syringe connector to ease clinical field access, and options of disposable and reusable devices to apply force to a standard syringe design, and a premix CaP cement. Experienced clinicians⁴ assessed the overall system characteristics and handling as being better than current practice by means of a workshop. See Fig. 1 a. In hip fracture lag screw augmentation a key need is the ability to inject through the lagscrew cannulation.

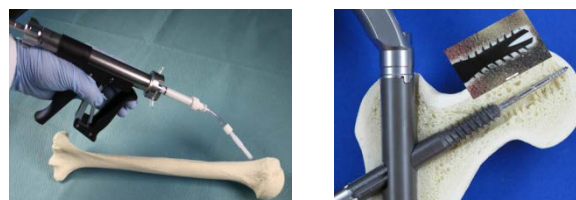


Fig. 1 a) Proximal Humerus, b) Proximal Femur
A special cannula was developed that replaced the guide wire and enabled cement to be delivered both safely and effectively at the right bony target area. The flexible coupling and the start stop characteristic of the CaP cement were also assessed as being advantageous over current clinical practice. See fig 1 b.

DISCUSSION & CONCLUSIONS: The collaborative approach where each partner brings specific expertise demonstrates a new business model. The unmet clinical need for augmented fixation in poor bone is substantial. The feasibility of the solutions was demonstrated in-vitro in a clinician workshop setting and surgeons confirmed that the solutions were potential advances on current clinical practice. It is intended to supply a rescue/revision kit per clinical indication. This will comprise: specialty CaP delivery screws of different lengths/diameters together with flexible connector and specialty cannula and injectable pre-mix cement capable of start stop behaviour. The next stage in the project is to complete these indication packages for clinical evaluation.

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Enhanced bone augmentation by using ultraviolet light mediated photofunctionalized titanium implant and mesh in rat femur model.

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INTRODUCTION: Guided bone surgery using thin titanium materials, e.g., titanium mesh, faces many challenges. Ultraviolet (UV) light -mediated photofunctionalization has earned a considerable attention to enhance biological capability of dental implants. It is unknown, however, whether and how photofunctionalization is effective in enhancing bone regeneration. This study examined the effects of UV treatment on bone regeneration with titanium implant and mesh in rat femur vertical augmentation model.

METHODS: Acid etched titanium implant that was 4 mm of length and 1 mm of diameter and titanium mesh products were used in this study. The materials were autoclaved and stored under dark ambient conditions for 4 weeks. UV treatment was performed by a combination of UVA and UVC for 15 min. A vertical bone augmentation model using the implant and mesh was created in rat femur bone by placing 2 mm of the 4 mm implant. The titanium mesh covered upper side of the implant that was exposed outside of femur. The samples were taken 24 days after surgery. Bone formation between the implant and mesh and the osteointegration were evaluated.

RESULTS: Bone volume between the photofunctionalized implant and mesh was significantly higher than the untreated on 24 day after surgery. The newly formed bone in photofunctionalized group completely filled the space that was surrounded by the implant and mesh, whereas the space was not filled with the new bone in untreated group. The mechanical strength analysis showed the connection between the implant and mesh in photofunctionalized group was 2-times greater than that in untreated group.

DISCUSSION & CONCLUSIONS: The results from in vivo studies collectively demonstrated that UV photofunctionalization of titanium mesh is effective in enhancing its osteoconductivity of both the implant and mesh, which resulted in the increased bone formation that could fill the space between titanium materials.

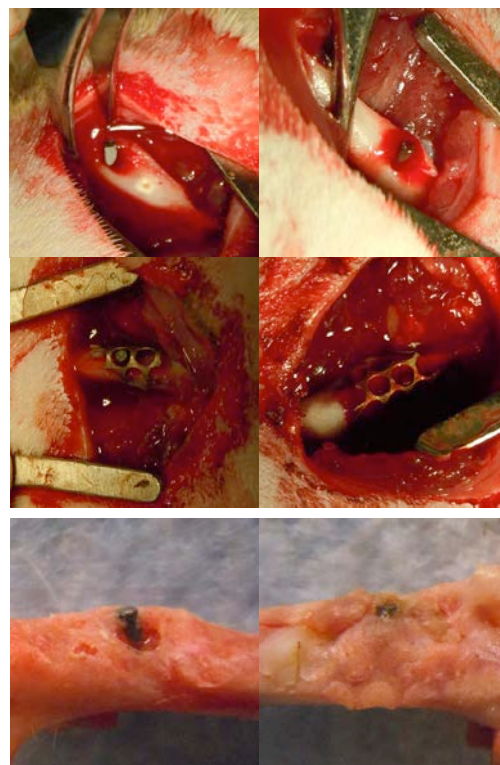


Fig. 1: Images of bone formation in untreated (left side) and UV-treated (right side) implant and mesh. UV-treated implant attracts blood (upper and middle) and is completely covered with newly formed bone (lower).

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Nanotomographic and compression analysis of β -TCP granules prepared with different formulations

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INTRODUCTION: Among biomaterials used for bone filling, β -TCP is known to be suitable in non-weighting bones and particularly in dental implantology, oral and maxillofacial surgery [1]. When β -TCP granules are placed in a bone defect, they occupy the void 3D volume. Little is known about the 3D arrangement of the granules which depends of the nature and the size of the granules themselves [2].

METHODS: The aim of this study was to examine the 3D arrangement of porous β -TCP granules prepared with different concentrations of powder in the slurry. It is an important factor which will allow a better invasion of cells, biological fluid and vascular sprouts in the grafted area. Six types of β -TCP granules were prepared with 10, 11, 15, 18, 21, and 25g of β -TCP powder in distilled water. Granules were prepared by the polyurethane foam process [3; 4]. Single granule of the different types were analyzed by nanocomputed tomography (NanoCT) on a Nanotom (GE) and compared with scanning electron microscopy. Compression analysis of the different powders was also measured with an Instron machine.

RESULTS: The outer and inner structure of the granules was shown by nanoCT which evidenced the macroporosity and microporosity (Fig.1). Macroporosity increased as an inverse relationship with powder concentration in the slurry. An internal porosity due to the sublimated polyurethane foam was present on all granules cross-sections. However, the microporosity due to grain joints was only evidenced by SEM. On 2D sections, the granules presented a heterogeneous aspect which corresponded to different mineralization degrees of the sintered powder grains.

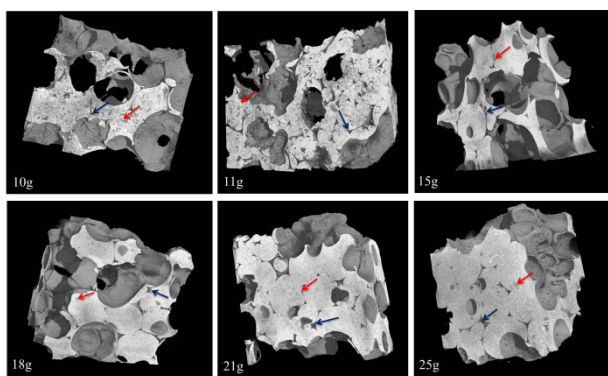


Fig. 1: NanoCT images of the different types of granules

Mechanical tests were highly dependent on the number and the spatial distribution of macropores. The granules with the lower amount of β -TCP were the most porous and were the more friable. On the contrary, the 25g denser granules exhibited the highest compressive energy (Fig.2).

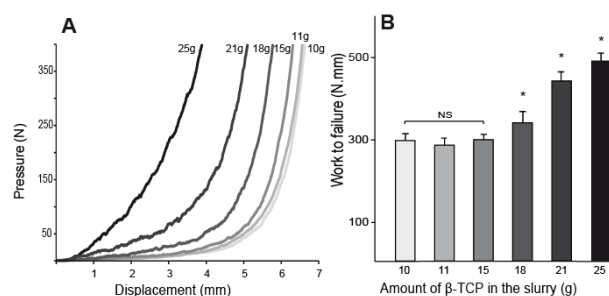


Fig. 2: Compressive analysis of β -TCP granules

DISCUSSION & CONCLUSIONS: For obtaining an improved formulation, there is a need to balance between a sufficiently porous material (which will facilitate osteoconduction) and sufficient biomechanical properties of the stacked granules in the grafted site.

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In Vitro evaluation of collagen reinforced calcium phosphate bone cementsI Palmer¹, J Nelson², W Schatton³, NJ Dunne¹, FJ Buchanan¹, SA Clarke⁴¹ School of Mechanical & Aerospace Engineering, Queen's University Belfast (QUB), Northern Ireland (NI), ² School of Biological Sciences, QUB, NI, ³ Klinipharma GmbH, Frankfurt am Main, Germany, ⁴ School of Nursing & Midwifery, QUB, NI

INTRODUCTION: Calcium phosphate bone cements (CPCs) are used in a wide range of clinical applications. Their bioactivity can potentially induce *in vivo* responses such as osteoclastic resorption and promotion of natural bone remodelling and ingrowth. Due to concerns regarding their mechanical properties, the incorporation of fibres into CPCs has been investigated. Incorporation of bovine collagen significantly improves compressive modulus and fracture toughness of CPCs without compromising setting time or injectability¹. Similar improvements have also been seen from incorporation of marine derived collagen fibres². Due to the role of collagen in bone function and formation, it is hypothesised that incorporating collagen into CPCs will also result in biological benefits.

METHODS: An α -tricalcium phosphate based CPC (α -TCP-CPC) was formulated from α -TCP powder and 5 wt% Na₂HPO₄ in dH₂O at a liquid to powder ratio (LPR) of 0.35 mL/g. Collagen-CPC composites, both bovine (BC-CPC) and marine (MC-CPC), were produced by adding 1 wt% collagen into the powder and liquid phases respectively. A further CPC (Merck-CPC) based on a commercial formulation³ and a commercial injectable PMMA bone cement (Vertebroplastic® Radiopaque Resinous Material, DePuy) were used as controls.

In vitro analyses using human bone marrow stromal cells (hBMSCs) were performed to assess cytotoxicity, cell proliferation and differentiation using lactate dehydrogenase (LDH), PicoGreen® and alkaline phosphatase (ALP) activity assays respectively.

RESULTS: Cell death on each cement was relatively low (<15 %). Collagen incorporation increased cytotoxicity but only to the extent observed for other commonly used biomaterials such as hydroxyapatite⁴. hBMSC proliferation occurred on all cements (Figure 1). Scanning electron microscopy confirmed these findings, with cells adhering and proliferating on each of the cement formulations. The most notable difference between cements was in the induction of osteoblastic differentiation (Figure 2). Large increases in ALP activity were observed, and was significantly lower on PMMA compared to the CPCs. ALP activity was similar on the collagen-CPCs at each time point; however, by Day 21 ALP activity was significantly higher on both unaugmented CPCs when compared to BC-CPC.

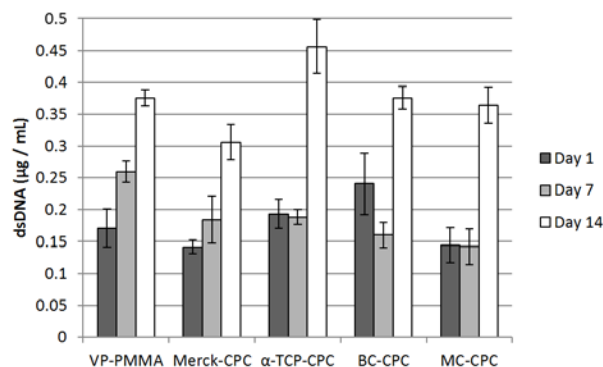


Figure 1: dsDNA concentration.

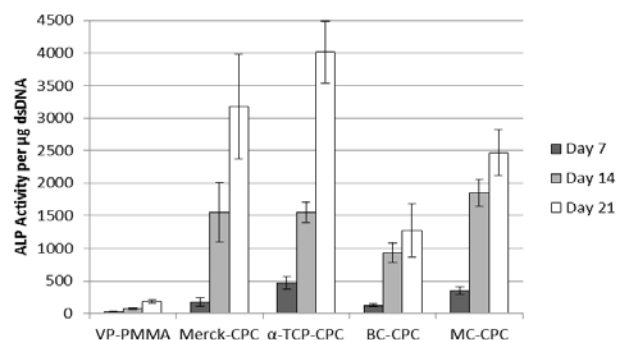


Figure 2: ALP activity per µg dsDNA.

DISCUSSION & CONCLUSIONS: Marine derived collagen has little influence on the *in vitro* biological properties of α -TCP-CPC; however, since its incorporation significantly improved the mechanical properties, it may still prove a suitable alternative to some commercially available bone cements.

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Cavuplasty procedure reduces cement extravasation during PMMA-augmentation of osteoporotic vertebral body fracture. A prospective randomized clinical trial

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INTRODUCTION: Prior to cement injection for vertebroplasty, an irregular cone shaped cavity was created in vertebral body through a special instrument, a procedure that is termed as Cavuplasty. The aim of this procedure is to reduce the injection pressure of PMMA with subsequent less extravasation of cement from vertebral body. The purpose of this study was to investigate whether cavuplasty is superior to vertebroplasty with regard to the rate of cement extravasation.

METHODS: 36 Patients with 42 stable osteoporotic vertebral body fractures (type A1, AO classification) in thoracolumbar area (Th4-L5) who were recommended for vertebral body augmentation were randomly assigned to either conventional vertebroplasty (n=18; 15f/3m; Ø 68.8 years old, 21 vertebral bodies) or cavuplasty (n=18; 17f/1m; Ø 72.3 years old, 21 vertebral bodies) treatment. For cavuplasty a special instrument (Cemento EC, OptiMed global care) was introduced through the needle into the vertebral body prior to cement injection.

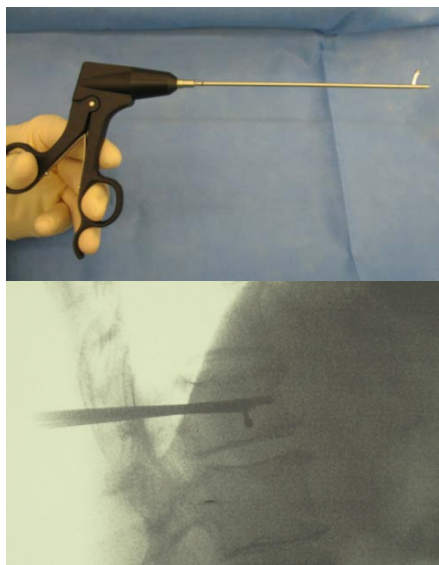


Fig. 1: Cemento EC created a cavity in the vertebral body by opening the tip in an alternatively rotating fashion prior to injection of PMMA.

By opening of the tip of the instrument through manipulation of the lever with a scissor grip bone tissue was compressed aside. This was repeated by alternatively rotating and manipulating the instrument in a circular manner until a cone-shaped cavity was created. Cement leakage and site of extravasation was detected by a postoperative CT-scan according to YEOM-classification. Clinical outcome expressed as pain reduction was evaluated by VAS preoperatively as well as 6 and 12 weeks postoperatively. The statistical analysis of cement leakage was performed by using the four field method and the chi-square test.

RESULTS: The average duration of the procedure was 31.5 minutes for vertebroplasty and 37 minutes for cavuplasty. The average volume of injected cement in each vertebral body was 3.26 (± 0.8 SD) ml for vertebroplasty and 3.32 (± 0.87 SD) ml for cavuplasty. Cement leakage occurred significantly less in cavuplasty group. Of the 21 vertebral bodies treated with vertebroplasty 14 showed leaks of cement compared to the cavuplasty group in which cement leakage was detected in 3 vertebral bodies ($p=0.0013$). None of these cement leakages caused symptoms.

Table 1: Rate of PMMA leakage by two augmentation procedures for osteoporotic vertebral fracture

PMMA	Cavuplasty	Vertebroplasty
Yes	3	14
No	18	7

DISCUSSION & CONCLUSION: Occurance of cement leakage is very common during vertebral body augmentation which may result in life threatening complication. Cavuplasty is a safe, cost efficient, easy to use cement augmentation technique that reduces cement leakage compared to conventional vertebroplasty procedure.

Effect of powder-to-liquid ratio and needle size on the extrusion of gray and white Portland cement for dental root filling application

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INTRODUCTION: Portland cement (PC), is used worldwide in the construction industry¹ and recently has also been approved for clinical use. Mineral trioxide aggregate (MTA), a PC-based root filling material, available in either gray or white form, has been widely used as repair material in endodontics, however it is difficult to deliver clinically due to its granular and sand-like consistency. Other problems associated with MTA are the high cost of the cement and requirement for specially designed delivery devices². Thus, the aim of this study was to evaluate a simple method for placing PC using medical stainless steel needles and to compare the extrusion (I) of white (WPC) and gray PC (GPC).

METHODS: WPC or GPC containing 20% Bismuth oxide was mixed with distilled water at powder-to-liquid ratios (PLR) of 3, 3.5 and 4.0g/ml, then placed into a 5ml syringe using needles with inner diameter of 0.69mm (19 gauge (19G)) to deliver the pastes. The cement was extruded using a universal mechanical testing machine with a cross-head speed of 30mm/min, which was continued until either the entire paste was extruded, or a maximum force of 100N was reached. The percentage of paste extruded was determined as the mass of the extruded paste/the original mass of the paste inside the syringe. Compressive strength (CS) and porosity (P) of the WPC/GPC samples were also evaluated.

RESULTS: The extrusion of PC is shown in Figure 1. GPC was practically fully extruded at a PLR of 3g/ml, PLRs of 3.5 and 4.0g/ml were still injectable, however, the percentage of extruded cement decreased with increasing PLRs. WPC showed markedly reduced extrusion at a PLR of 3g/ml, PLRs of 3.5 and 4.0g/ml were not injectable at all. CS and P were inversely proportional, the increase of the water content led to decreased CS and increased P values for both GPC and WPC.

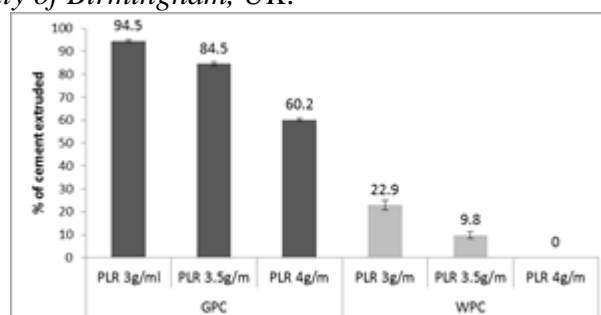


Fig. 1: Percentage of extruded GPC and WPC using 19G stainless steel needles.

Fig. 2: Influence of PLRs on the compressive strength and porosity of GPC and WPC.

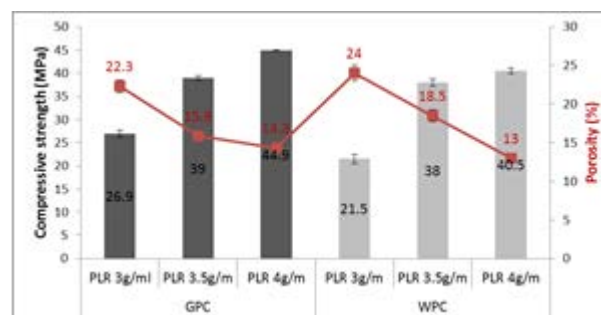


Fig. 2: Influence of PLRs on the compressive strength and porosity of GPC and WPC.

DISCUSSION & CONCLUSIONS: Although GPC and WPC showed similar mechanical behaviour, the injectability of WPC was markedly reduced. This could have been influenced by the size of the cement particles. WPC is a very fine powder with smaller particles when compared with GPC. The smaller the particles, the more water will be retained in the interparticulate spaces by surface tension, therefore, water is rapidly absorbed making the mixture less fluid and difficult to inject. Only GPC could be made injectable in this study and 19G needles were demonstrated to be suitable for delivery of this MTA.

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Assessment of drillability of CERAMENT™ | BONE VOID FILLER

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INTRODUCTION: The ability to drill through a bone graft substitute bears great relevance to orthopaedic hardware installation. It is desirable that the administered bone graft substitute should have the capacity of remaining stable during drilling and that the placement of a screw does not induce breaks or cracks in the material. The purpose of this study was to investigate the effects of drilling and screw insertion through an injectable synthetic bone graft substitute consisting of hydroxyapatite and calcium sulfate, CERAMENT™ | BONE VOID FILLER (CBVF). In vivo, the calcium sulfate component resorbs with time and is replaced by ingrowing bone [1]. CBVF is indicated to be placed into bone voids or gaps in the skeletal system, not intrinsic to the stability of the bony structure.

METHODS: The experiments were carried out similarly to the method described by Granberry et al. [2]. An open cell rigid foam from SAWBONES® was used (density: 0.12g/cc), simulating osteoporotic cancellous bone. The foam was cut into blocks with dimensions 30 × 30 × 40mm. A ~12.5mm deep void was created at the top of the samples, using a bench drill, with a diameter of 12.8mm. The blocks were then fully immersed in 125mL of Ringer solution (prepared with deionized water, 0-0.1µS/cm) maintained at 37°C, to simulate physiological conditions. CBVF (BONESUPPORT AB, Sweden) was mixed according to instructions in the IFU and 2-3mL of paste were injected at 3min into the void (while the samples were immersed). 15 minutes after mixing, drilling was performed with a bench-drill, using a 3.9mm drill-bit and infiltrating the blocks to a depth of 25mm. Two drilling speeds were examined, 600 and 900 rpm as according to Bertollo and Walsh [3], in orthopaedics, speeds of <1000rpm are employed. An Ansis III screw (5.0×60mm, Stryker) was inserted straight after drilling. The sample was placed back into the solution and sectioning was performed after 2h. The drilled canal and the adjacent areas were examined for breaks and/or cracks optically and with a magnifier lamp.

RESULTS: CBVF was easily drilled and the material that accumulated at the top as a result of the drilling held together (Fig. 1c) and could be removed. The drilling channel maintained its shape and the screw was manually inserted. The screw went through the bone graft substitute and into the foam (Fig. 1d). In the cross-sections it can be seen that the area surrounding

the drilling channel is free of fractures (Fig. 1e&f). This was observed for both drilling speeds.

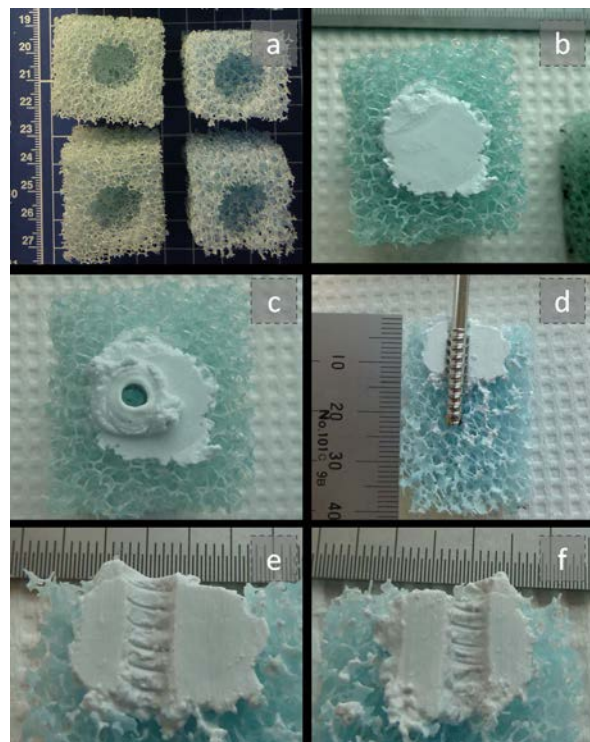


Fig. 1: a) SAWBONES® blocks with created voids, b) Void filled with CERAMENT™ /BVF, c) Drilled sample, d) Illustration of placement of screw through the cement, anchorage in foam, e & f) Cross-sections of drilling channel

DISCUSSION & CONCLUSIONS: This study shows that CERAMENT™ | BONE VOID FILLER can be drilled and a screw can be placed through the material without producing cracks. The results support the use of CBVF in non-load bearing applications where screws are anchored in good-quality bone.

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Real-time monitoring of transformation processes of biogenic calcium phosphate materials for bone tissue engineering applications

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INTRODUCTION: This study offers physico-chemical insights into biomineralization mechanism of hard tissue formation, by bringing into focus phenomena taking place in octacalcium phosphate (OCP) - dicalcium phosphate dihydrate (DCPD) - Simulating Body Fluid (SBF) system. It is known that bone has a hierarchical structure, its primary unit being based on the nucleation of calcium phosphates in nanoscale spaces organized within the supramolecular assembly of collagen fibrils. Therefore, controlled crystal nucleation and growth is a fundamental issue in materials chemistry applied to biological systems. In connection with this, the aim of this work was *in situ* investigation of structural transformations happening to OCP-based systems, being of great interest for biomedical applications due to biocompatibility, osteoconductive and possible osteoinductive properties.

METHODS: The Energy Dispersive X-Ray Diffraction (EDXRD) technique was applied to follow structural transformations in real time. For each sample, a sequence of diffraction patterns, collected as a function of scattering parameter and of time (a 3D map), was obtained (Fig. 1). Together with phase transformations (initial-intermediate-final phases), amorphous-into-crystalline conversion, i.e. the primary and secondary crystallization processes, was monitored *in situ*, and the characteristic crystallization times for the phases of interest were deduced. Long-time *in situ* EDXRD measurements, performed in this work, demonstrated that hardening process of calcium phosphate bone cements is much more complex than expected [1-3].

RESULTS: The obtained experimental results allow us to conclude that when only OCP is present in SBF, the formation of carbonated hydroxyapatite takes place, whereas for the OCP-DCPD (30%) system in SBF, less kinetically favourable hydroxyapatite (HA) is formed. The additional component (DCPD) influences significantly the structural behaviour of hydroxyapatite, acting as the HA crystallization inhibition agent [1].

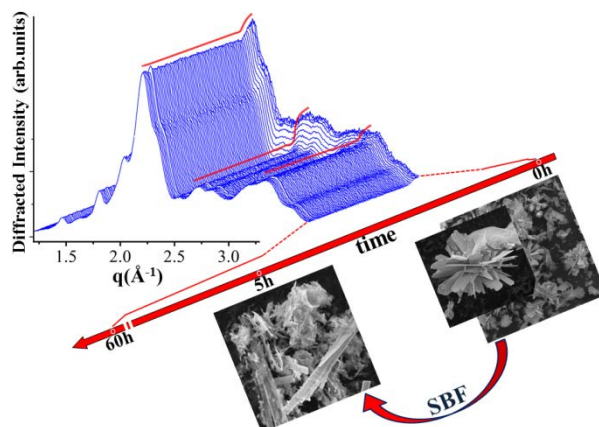


Fig. 1: 3D Map of the EDXRD patterns collected upon the OCP-DCPD-SBF system. Diffraction data are complemented with the SEM results.

DISCUSSION & CONCLUSIONS: In order to control the HA crystallization in a biomineralization processes, it is of considerable biomedical value to know how additives can affect the crystallization rates. In this study, the crystallization dynamics of several biogenic calcium phosphate materials was studied in the presence of additive molecules. The co-operative effect of the OCP-DCPD combination in SBF, leading to the HA formation, was investigated. By means of the OCP-DCPD cooperation approach, allowing to control the HA crystallization, calcium phosphate based materials can be suitably modified for implant-use in bone replacement or tissue engineering. In particular, for the OCP-based cements (OCP phase precipitates very rapidly into HA), the observed inhibition effect of DCPD can be applied for novel self-setting calcium phosphate cement formulations.

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Comparison of three experimentally-induced models of intervertebral disc degeneration in rabbits.

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INTRODUCTION: This study was designed to compare three experimental methods (aspiration¹, enzymatic treatment² and laser³) to induce intervertebral disc (IVD) degeneration in rabbits. The ultimate goal was to help scientists choose the most suitable model of IVD degeneration to further investigate *Nucleus pulposus* regenerative therapies.

METHODS: One-year-old New Zealand white rabbits were used (n=30). MRI of each rabbit lumbar spine was performed to exclude spontaneously degenerated IVD from further surgical procedure. IVD were identified under fluoroscopy and the lesions were induced according to the following protocol: needle aspiration, laser and enzymatic treatment (hyaluronidase). Untreated IVD were used as control. IVD degeneration was followed at day 7, 30, 60 and 90. Discal height was measured with X-ray radiographs and T2-weighted signal intensity (T2wsi) and Pfirrmann's grading were determined on MRI images. Histological IVD degeneration was evaluated according to the Boos' scoring⁴.

RESULTS: After 90 days and even much sooner, aspiration, laser and enzymatic treatments induced a gradual and significant degeneration of IVD with a decrease in IVD height (data not shown) and T2wsi (Fig.1).

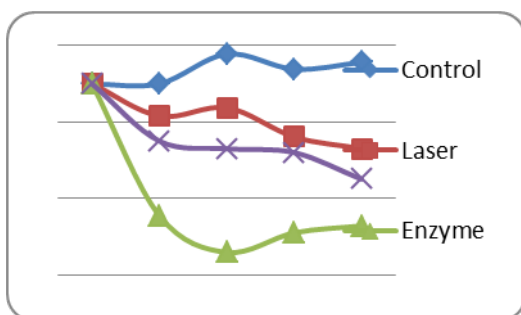


Fig. 1: Decrease in T2-weighted signal intensity of IVD after treatment (%)

Contrarily to laser, enzyme and aspiration induced a significantly more pronounced degeneration. The degeneration induced by enzymatic treatment is faster. Indeed, 3 months after surgical procedure, the decrease

in discal height was about 12 %, 19% and 50 % in laser, aspiration and enzyme groups respectively. The decrease in T2wsi was 17%, 25% and 37% in the same

conditions. Histological analysis and Boos'scoring (Fig.2) confirmed the gradually decrease after laser treatment contrarily to aspiration and enzyme treatments.

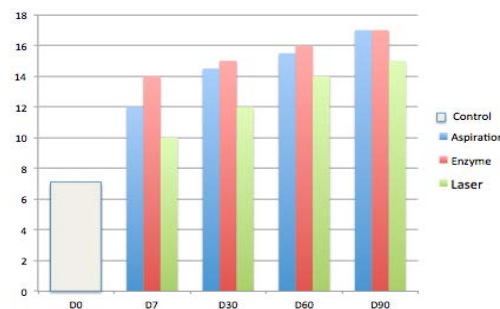


Fig. 2: Boos' scoring after laser, aspiration and enzyme treatments.

DISCUSSION & CONCLUSIONS: Compared with control, the 3 treatments induce a significant IVD degeneration that is more prominent and rapid in enzyme and aspiration groups. Even if the mechanisms are still poorly understood, laser induces a progressive degenerative process, which is consistent with the spontaneous degeneration in human. This data confirm the relevance of laser-induced IVD degeneration and should be considered as a powerful model in animal studies of regenerative medicine.

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Monetite resorption of mouse bone marrow macrophage derived osteoclasts

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INTRODUCTION: Calcium phosphate cements (CPC) are interesting as bone grafting materials due to their similar composition to bone. The group of acidic CPCs; brushite and monetite are of special interest since these materials are resorbable *in vivo*. The resorption can be divided into three different routes; chemical dissolution, macrophages engulfing particles and osteoclastic resorption. Although acidic CPCs have been extensively studied for more than a decade, few studies have focused on the osteoclastic resorption thereof. Moreover, it has recently been suggested that monetite have superior biological properties compared to the hydrated form, brushite¹. Therefore this study was designed to evaluate the resorption of monetite by bone marrow derived osteoclasts.

METHODS: A premixed calcium phosphate cement² consisting of β -tricalcium phosphate, monocalcium phosphate anhydrous and glycerol was prepared. The cement paste was moulded and set at 100 % humidity at 37 °C. After several PBS washes the pieces were ready for cell seeding. Bone marrow derived osteoclasts cells were obtained from femur and tibia of 3 weeks old mice. The marrow cavities were flushed and red blood cells lysed prior expansion with macrophage colony stimulating factor (M-CSF). Adherent cells were seeded on monetite cement. To initiate osteoclast differentiation receptor activator of NF- κ B ligand (RANKL) was added in combination with (M-CSF). After 7 days cell media was collected and Ca^{2+} concentration was analysed by a colorimetric assay. Cells were fixed for SEM and stained for tartrate-resistant acid phosphatase (TRAP), as an indication of osteoclast formation.

RESULTS: TRAP positive cells could only be detected in wells treated with both M-CSF and RANKL and the TRAP positive cells are large and multinucleated, characteristic for osteoclasts (fig. 1A). In figure 1B the SEM picture shows a cell sunk into the monetite surface, forming a resorption lacuna. Moreover, the Ca^{2+} measurements revealed significantly higher concentrations in wells with TRAP positive cells. The concentration was compared to wells with TRAP negative cells (only treated with M-CSF) and to wells without any cells. No difference in Ca^{2+} concentration could be detected between the two latter groups.

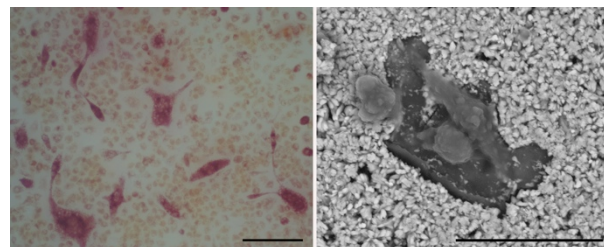


Fig. 1: Cells have been treated with M-CSF and RANKL for 7 days. A) TRAP positive cells on monetite cement. Bar = 100 μm . B) SEM picture of osteoclast in a resorption lacuna. Bar = 30 μm .

Table 1. Ca^{2+} concentration in cell media of monetite samples with no cells, cells treated with M-CSF or M-CSF + RANKL. * indicates $p < 0.001$.

	No cells	M-CSF	M-CSF + RANKL
Ca^{2+} [mM]	2.6 ± 0.3	2.3 ± 0.4	$3.6 \pm 0.3^*$

DISCUSSION & CONCLUSIONS: The results from TRAP staining, SEM and Ca^{2+} concentration suggest that actively resorbing osteoclast have formed on the monetite cements. This is in agreement with a previous study by Grossardt *et al.*, although they used the macrophage cell line RAW 264.7 with limited resorption³. *In vitro* studies of osteoclast resorption of monetite will be a valuable tool for optimizing cement properties regarding the resorption rate. Until now the focus have been on osteoblastic response to monetite, however, the authors believe that there is much to explore regarding the osteoclast response.

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Ready-to-use α -TCP cements based on cationic exchange

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¹ [RMS Foundation](#), Bettlach, CH

INTRODUCTION: Calcium phosphate ceramics have been used successfully as synthetic bone void fillers for many years. α -TCP (α -Ca₃(PO₄)₂) is of particular interest because it hydrates when mixed with water, and the slurry undergoes a setting reaction forming a solid cement body of Ca-deficient hydroxyapatite (CDHA, Ca₉(HPO₄)(PO₄)₅OH). α -TCP is therefore a common constituent in hydraulic bone cements. Certain elements dissolved in the liquid phase of the paste inhibit the nucleation of CDHA and suppress the hydration reaction. The effect can be reversed by increasing the concentration of Ca²⁺ in the solution. Based on these cation exchange processes we developed a bone cement using an aqueous α -TCP paste with a nucleation inhibitor, and an activator solution. In the present study we analyzed the inhibitory potential of various ions and the effect of activator concentration on the setting time of the aqueous α -TCP paste.

METHODS: α -TCP raw material was prepared in a solid-state reaction from CaHPO₄ and CaCO₃. The powder blend was heated at 1350 °C, quenched, milled, and annealed at 500 °C for 24 h.

The long-term stability of α -TCP pastes was tested in a real-time shelf life test at 21 °C for up to 8 weeks. Pastes were mixed from 2.0 g α -TCP + 1.0 ml MgCl₂ solution (0.1 and 0.5 M). After storage, the pastes were washed with ethanol and dried, the α -TCP content was measured by XRD, and the particle surface was analysed by SEM.

To initiate the setting reaction, pastes were prepared from 2.0 g α -TCP + 0.8 ml solution containing 0.1 M MgCl₂, SrCl₂, BaCl₂, ZnCl₂, CuCl₂, or NiCl₂. BaCl₂ was also tested in 0.01 M. 0.2 ml of 0–4 M CaCl₂ activator solution was admixed. The progress of the setting reaction was monitored by micro-calorimetry at 37 °C for up to 2 weeks.

RESULTS: The shelf life test revealed that pastes containing 0.1 or 0.5 M MgCl₂ were stable up to 8 weeks, no precipitation was observed on the particle surface (Figure 1). Control samples prepared with H₂O completely reacted within 3 weeks.

Calorimetry data showed that all elements except Zn²⁺ fully inhibited the setting reaction. The observed inhibition potentials differed vastly, requiring no CaCl₂ (Zn²⁺, Cu²⁺), low (Mg²⁺, Sr²⁺), or high (Ba²⁺) CaCl₂

concentrations in the activator solution to initiate the reaction (Table 1). CaCl₂ concentrations higher than the minimum amount resulted in faster setting, approaching the time observed with inhibitor-free formulations. Inhibition by Ni²⁺ was too effective to be activated with CaCl₂ concentrations tested in this study.

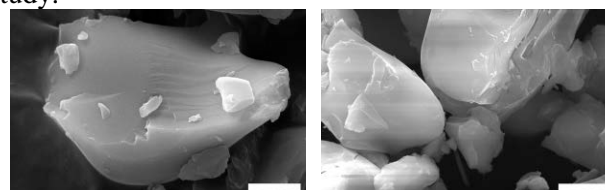


Fig. 1: SEM images of the α -TCP particle surface before (left) and after 8 weeks incubation in 0.1 M MgCl₂ solution (right). Scale bar = 2 μ m.

Table 1. Minimum CaCl₂ concentration required to initiate the setting reaction during the 2 weeks of observation. Mixing ratio: 0.8 ml inhibitor + 0.2 ml activator solution. Total LPR: 0.5 ml/g.

Inhibitor sol.	Activator sol.	Ca ²⁺ :M ²⁺
0.1 M ZnCl ₂	H ₂ O	-
0.1 M CuCl ₂	H ₂ O	-
0.1 M SrCl ₂	0.2 M CaCl ₂	1:2
0.1 M MgCl ₂	0.4 M CaCl ₂	1:1
0.1 M BaCl ₂	2 M CaCl ₂	5:1
0.01 M BaCl ₂	0.2 M CaCl ₂	5:1
0.1 M NiCl ₂	> 4 M CaCl ₂	> 10:1

DISCUSSION & CONCLUSIONS: Our data shows an inverse correlation of the inhibitory potential of various cations M²⁺ with their defect formation energies in the apatite crystal structure in aqueous solution, and with differences in ionic radii¹. Matsunaga et al. reported the following sequence of compatibilities with the apatite crystal structure: Ca²⁺ > Zn²⁺ > Mg²⁺ > Ba²⁺. This sequence indeed inversely correlates with the concentration of CaCl₂ required to compensate the inhibition. Our study demonstrated that incompatible cations M²⁺ in solution inhibit the nucleation of CDHA. Injecting activator solution increases the Ca²⁺:M²⁺ ratio and reduces the relative concentration of incompatible cations, eventually enabling the nucleation of CDHA crystals.

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The Broad-spectrum Antimicrobial Activity and Biocompatibility of the Mesoporous Silica Nanoparticles Encapsulated with Silver Nanocrystals

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INTRODUCTION: The Mesoporous Silica Nanoparticles Encapsulated with Silver Nanocrystals (Ag-MSN) material has an excellent biocompatibility and mechanical capacity. It presents an ability to load and control the release of drugs, therefore, it is a potential antibacterial coating for bone substitute materials. In this study we examined the Ag-MSN material's antibacterial capacity, biocompatibility and its Ag ion release characteristics in vitro to evaluate the feasibility for the use of this material to treat the bone defect after chronic osteomyelitis surgery.

METHODS: The ex vivo antibacterial effect against *Staphylococcus aureus* (*S. aureus*, 25923, ATCC), *Escherichia coli* (*E. Coli*, 25922, ATCC), *Pseudomonas aeruginosa* (*P. aeruginosa*, 9027, ATCC), *Bacteroides fragilis* (*B. fragilis*, 25285, ATCC), and *Candida albicans* (*C. albicans*, 10231, ATCC) of different concentration of Ag-MSN suspension was detected by Standard broth dilution method and OD600 Bacterial growth curve assay. To verify the Ag ion release curve and assess the material's Ag release characteristics, the Ag-MSN (400/200/100/50µg/ml) was added into simulated body fluid (SBF) and the Ag concentration was determined by flame atomic absorption spectrometry (FAAS) at different time points. The CCK8 assay and alkaline phosphatase (ALP) activity test were also used to test the biocompatibility of different concentration of Ag-MSN and MSN suspension while Osteoblast-like MG63 cells were cultured.

RESULT : We confirmed that the Minimum Inhibitory Concentration (MIC) of Ag-MSN material against *S. aureus*, *E. Coli*, *P. aeruginosa* and *B. fragilis* were below 200µg/ml, and the Minimum Bactericidal Concentration (MBC) were at 400,200,200,200µg/ml, respectively. The middle concentration of Ag-MSN suspensions could significantly inhibit bacterial growth compared with the control group after 12 hours, however, the Ag-MSN suspension did not exhibit obvious sterilization effect on *C. albicans*, and could only inhibit its growth at 400µg/ml concentration. We observed a similar trend of Ag ion release in different concentration groups of Ag-MSN suspensions that the Ag ion concentration of all groups reached the peak

after soaking for 6 hours, and stabilized after soaking for 3 days, which had no significant difference compared to soaking for 14 days. Ag ion's biological safety Concentration was reported to be 54mg/L,

whereas its minimal inhibitory concentration was 0.5mg/L—0.05mg/L, the Ag ion in 400,200 and 100µg/ml concentration groups were always between these two levels during the experiment. The CCK8 assay indicated that the Ag-MSN and MSN didn't restrain the proliferation of MG63 cells. The results of alkaline phosphatase (ALP) activity test and the expression of osteogenic marker genes, such as ALP and osteocalcin, demonstrated that the differentiation of MG63 cells might be enhanced by Ag-MSN and MSN.

DISCUSSION & CONCLUSIONS: Ag-MSN material has a significant bactericidal effect against *S. aureus*, *E. Coli*, *P. aeruginosa*, and *B. fragilis* in vitro, also it presents an inhibitory effect against *C. albicans*. These data demonstrate that the Ag-MSN material has a good biocompatibility and sustained release quality in SBF, which makes it a potential candidate of bone substitute antibacterial coating material against chronic osteomyelitis.

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Key Word: Mesoporous Silica Nanoparticle ; Antibacterial coating ; Chronic osteomyelitis ; Ag ion;

An injectable hyaluronan hydrogel with microcavities for cartilage tissue engineering

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INTRODUCTION: The injectable materials have attracted increasing attention in tissue repair. The minimally invasive nature of injectable scaffolds can reduce discomfort for patients; furthermore, injectable scaffolds also possess the ability to fill the irregular defects.^[1] The fabrication of pure hyaline cartilage is a great challenge for cartilage repair and the living hyaline cartilage graft has been developed by phase transfer cell culture in microcavitary alginate hydrogel.^[2] However, the uncontrollable and unpredictable degradability of alginate hydrogel *in vivo* dramatically limited its clinical application. Hyaluronan (HA) is an important cartilaginous extracellular matrix (ECM) component, which can bind specifically to the surface receptors on chondrocytes, regulating cell motility, proliferation and differentiation.^[3] Here, the microcavitary injectable hyaluronan hydrogel was prepared via *in situ* chemical cross-linking of glycidyl methacrylate-modified hyaluronan (HA-GMA).^[4] The cytocompatibility of the hydrogel was studied.

METHODS: *Fabrication of construct:* Porcine chondrocytes were extracted from pig (five months' old) knee articular cartilage and cultivated in chondrocyte culture medium (Dulbecco's Modified Eagle Medium (DMEM) supplemented with 0.4 mM proline, 10 mM HEPES, 50 mg/L Vitamin C, 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 0.1 mM nonessential amino acids) in the humidified incubator at 37 °C with 5% CO₂. Chondrocytes at passage one (10 million cells per mL) and gelatin microspheres with the sphere size of 150-180 μm (0.2 gram per mL) were suspended together in HA-GMA precursor solution (4%, w/v), and placed on ice cubes. Ammonium persulfate (APS, 0.5%, w/v) and N,N,N',N'-tetramethylethylene diamine (TEMED, 0.1%, v/v) were added to the solution and mixed thoroughly with a pipette. The mixture solution was injected into cylindrical molds (diameter 4 mm, height 3 mm) and placed in 37 °C incubator for 5 minutes.

Cell viability: Live/Dead assay was used to investigate the viability of chondrocytes in construct after 24 hours encapsulation. Thin slices of the construct were incubated in Live/Dead assay dye solution for 30 minutes, and then observed under fluorescence microscope, where the live and dead cells fluoresced green and red respectively.

RESULTS: As shown in Fig. 1, the cavity was created in the construct, and the chondrocytes retained their round morphology and presented the high viability after 24 hours culture.

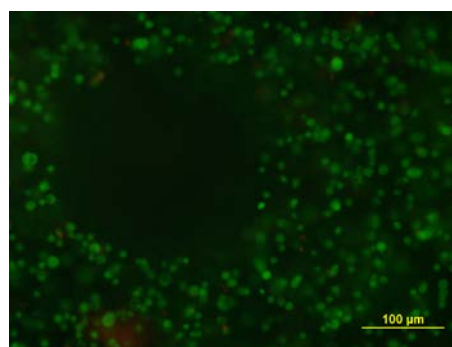


Fig. 1: Fluorescent image of chondrocytes encapsulated in construct.

DISCUSSION & CONCLUSIONS: The successful creation of the cavities in construct is beneficial to cell proliferation and the secretion of cartilage specific ECM, collagen type II and proteoglycans, due to the “edge flourish” phenomenon,^[2] and the high cell viability indicates the fabrication procedure of the construct is cytocompatible. This injectable hydrogel is a potential scaffold for cartilage tissue engineering.

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Influence of the shape of glass beads on the injection properties of β -tricalcium phosphate – glass beads – water pastes

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INTRODUCTION: Calcium phosphate cements and pastes are of great interest in non-invasive surgeries. However, when they contain granules, their rheological and injection properties are poor. Recently, our group tried to get a better understanding of this phenomenon by looking at the injectability of model pastes composed of β -tricalcium phosphate powder (β -TCP), water and spherical glass beads (100-400 μm) [1]. Since most granular bone graft substitutes are not spherical but angular, this study aimed at determining the influence of glass bead shape on the injectability of β -TCP – glass beads – water pastes.

METHODS: β -TCP powder from Sigma-Aldrich (No 2128, Steinheim, Germany) with a density of 3.1 g.cm^{-3} and a d_{50} of $1.8 \pm 0.4 \mu\text{m}$ was mixed with demineralized water to form a paste with a liquid-to-solid ratio of 0.45 ml/g. Spherical (Spheriglass CP01 ref. 2024 and 1619, Potters Europe) and angular glass beads (ref. ST-145 and ST-60, Reidt GmbH & Co. KG, Stolberg, Germany) with a density of 2.5 g.cm^{-3} were used. CP01_2024 (called “SS”, as “small” and “spherical”) and ST-145 (“SA”, as “small” and “angular”) had a size between 106 and 212 μm , whereas CP01_1619 (“LS”, as “large” and “spherical”) and ST-60 (“LA”, as “large” and “angular”) had a size between 250 and 425 μm . Pastes were homogenised one minute on a vortex and filled in a 1 ml syringe (BD Luerlock, Beckton Dickinson, USA; inner diameter: 4.5 mm, opening diameter: 1.9 mm). The plunger was then inserted so that its lowest part reached the 1 ml graduation. The syringe was placed in a metallic cylinder to avoid deformation during pressing. Injection tests were performed with a Zwicki-Line Z5.0 (Zwick, Kennesaw, GA, USA) compression tests machine. Displacement rate was fixed at 0.4 mm/s and tests were automatically stopped when a 250 N load was reached. Injectability was defined as the weight percentage of extruded material. Median beads size was determined from optical microscopies and sphericity was calculated from the inscribed (d_{50_ins}) and circumscribed (d_{50_circ}) maximum diameters.

RESULTS: Fig.1 shows that about 80% of the pastes could be injected at low bead content. As observed in our previous study, the addition of a small bead volume fraction increased significantly the injectability. However, beyond a certain volume fraction, which was higher for smaller and spherical beads (20 vol.% for angular ones, compared to 40

vol.%), a rapid injectability drop was measured.

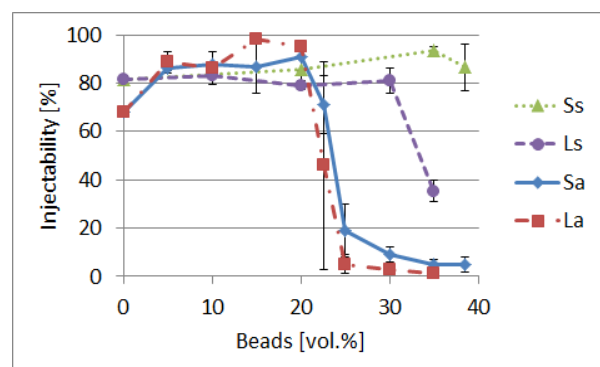


Fig. 1: Injectability results as a function of the bead content. The results with SS and LS were presented in [1].

Table 1. Measured beads size, sphericity and bulk density

Glass beads	d_{50_ins} [μm]	d_{50_circ} [μm]	Sphericity	Bulk D. [g.cm^{-3}]
SS	87 ± 55	87 ± 55	0.93 ± 0.06	1.43 ± 0.00
LS	282 ± 88	282 ± 88	0.95 ± 0.09	1.47 ± 0.00
SA	128 ± 31	232 ± 67	0.55 ± 0.11	1.08 ± 0.00
LA	285 ± 45	482 ± 72	0.58 ± 0.08	1.17 ± 0.00

DISCUSSION & CONCLUSIONS: Changing the shape of granules strongly reduced the injectability of pastes made of β -TCP powder, water and glass beads. Similar injectabilities were obtained for the two pastes composed of angular beads (SA and LA).

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Is Kiva implant advantageous to Balloon Kyphoplasty in treating osteolytic metastasis to the spine? A comparison of two percutaneous MIS techniques and a prospective randomized controlled short-term study

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INTRODUCTION: This announcement presents a prospective, parallel-group, controlled, comparative randomized study in order to compare the cement leakage rate and the efficacy for vertebral body restoration of balloon kyphoplasty (BK) versus Kiva novel implant with polymethylmethacrylate (PMMA) for treating osteolytic vertebral body metastasis.

Minimally invasively vertebral augmentation techniques with PMMA are mostly performed for treating osteoporotic compression fractures^{1,2}. The Kiva implant with PMMA offers better vertebral body restoration and less PMMA leakage than BK in osteoporotic fractures. No previous study compared leakage rate and efficacy for vertebral body restoration in traditional BK and Kiva with PMMA in osteolytic vertebral body metastases.

METHODS: This study examined 23 patients (71 ± 13 yr) with 41 osteolytic vertebral bodies, who received Kiva with low viscosity PMMA and 24 patients (70 ± 11 yr) with 43 vertebral body osteolyses, who were reinforced with BK and high viscosity PMMA. All osteolyses were graded as Tomita 1 to 3. Anterior vertebral body height ratio (AVBhr), posterior vertebral body height ratio (PVBhr), middle vertebral body height ratio (MVBhr), Gardner kyphotic deformity and PMMA leakage were measured and compared between the two groups. Visual Analogue Scale and Oswestry Disability Index were used for the functional outcome evaluation.

RESULTS: No patient survived after 3 months. Asymptomatic PMMA leakage occurred in 4 (9.3%) vertebrae in the BK group (2 to the spinal canal, in Tomita grade 3 osteolysis). Anterior vertebral body height ratio, posterior vertebral body height ratio,

both groups, Visual Analogue Scale and Oswestry Disability Index improved postoperatively (P< 0.001).

DISCUSSION & CONCLUSIONS: BK and Kiva provided equally significant spinal pain relief in patients with cancer with osteolytic metastasis. The absence of cement leakage in the Kiva group and the absence of neurological complication in the BK group leakages, reflects the safety of both augmentation techniques even in significant osteolysis. The lack of cement leakage in the Kiva cases, although low viscosity PMMA was used, increases this implant safety in augmenting severely destructed thoraco-lumbar vertebrae and sacrum from osteolytic metastasis.

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middle vertebral body height ratio and Gardner angle improved insignificantly similarly. In

Feasibility of a novel concept for making non-injectable biomaterials injectable

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INTRODUCTION: Bone graft biomaterials provide temporary or permanent replacement of bony structures that are either traumatised or degenerate through pathology. The broad spectrum of indications and diverse biomaterial properties^{1,2} often results in very challenging mixing and delivery processes. In particular DBM-like³, Putty-like and combination graft-materials are clinically very difficult to deliver to the operative site by an injection technique. To enhance the bone healing potential, other materials are frequently added by the surgeon e.g. bone chips, bone marrow aspirate or bone quality enhancing drugs. Frequently these are mixed by hand and then manually inserted into a surgical site by the surgeon. This time honoured method has the drawbacks of taking additional time, material is often wasted and may not be placed in the ideal site. In this paper we will demonstrate how it is possible to make some of these non-injectable biomaterials injectable.

METHODS: To overcome the clinical issues noted above the authors invented innovative tooling that enables efficient controllable and precise delivery of difficult to inject biomaterials. A key factor in delivery of such materials is to overcome the high pressures and shearing forces often generated during the loading and injection procedure in the operating room. The objective was to develop a device that would enable loading and injection of wide range of different material-configurations. The most promising solution developed was preloading the delivery tool with the premixed biomaterial and then to inject this into the operative site. A number of possible ways to achieve this were evaluated theoretically and one was selected as corresponding most closely to the clinical user needs.

For the practical evaluation in the test laboratory we utilised novel test-materials that simulated key-properties such as viscosity, stickiness, moisture and typical material particle sizes. The surface properties of the device and certain geometric parameters were found to be of critical importance.

The concept selected was approximately based on the Razor Clam shellfish in that two shell halves are joined by a longitudinal hinge mechanism as shown in Fig. 1.

In the final form of the idea the biomaterial is optimally formed into the shape of a cylinder. This is then transferred with the designed sleeve into the delivery device. The biomaterial is then injectable by a special plunger.

RESULTS: The device trials showed that this method formed a consistent intact cylinder of the biomaterial in typically less than 20 seconds. Whilst the cylinder of biomaterial could be injected with a minimum of effort, compared to conventional methods, there were considerable differences in pressure and shearing forces. So some material combinations could be injected by hand pressure whereas others required a delivery gun.



Fig. 1: Image of the initial shape (left) and the clinical desirable shape for injection (right).

DISCUSSION & CONCLUSIONS: The present feasibility study was designed to investigate the possibility making non-injectable biomaterials injectable. Our experimental results demonstrated, using the prototype tooling shown above, that some materials considered difficult or even un-injectable may be safely and effectively injected. It remains for a production version of the device to be made and evaluated clinically with a range of difficult to inject biomaterials.

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Palliative surgery prevents vertebral collapse and potential neural elements compression, restores vertebral body and improves pain and quality of life in Multiple Myeloma patients with spinal osteolytic lesions

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INTRODUCTION: This announcement presents a retrospective review that aims to present the functional outcome and survival rates following “palliative” surgery in 21 consecutive Multiple Myeloma (MM) patients who underwent a total of 25 surgeries in one institution and to review the relative literature.

MM is a systemic neoplasm of plasma cells and that affects 1-4 per 100,000 people per year, and is commonly associated with bone pain usually due to spinal and rib osteolyses in 70% of these patients [1-4]. Skeletal osteolyses is the most frequent cause of morbidity and mortality in patients affected by this pathology [5].

Percutaneous augmentation with PMMA in patients with osteoporotic and metastatic vertebral fractures has been a safe and successful procedure for vertebral body stabilization and axial pain reduction. In the case of spinal cord and cauda compression open decompression plus mesh cage stabilization has been successfully used in some cases. There are few short-term studies reporting vertebral augmentation for MM osteolytic spinal lesions.

METHODS: Between December 2004 and May 2012, 25 surgical procedures (percutaneous augmentation, hybrid fixation, and open circumferential decompression and stabilization) were performed by the authors for symptomatic vertebral body osteolysis and fractures in 21 consecutive patients with MM. Tomita classification for osteolytic lesion extension, and Karnofski scale plus ASIA neurological impairment scale and VAS pain scale were used. Survival analysis was performed.

RESULTS: All patients were followed for a minimum 6 months postoperatively. Karnofsky Index was improved from an average \pm SD, $66\% \pm 20\%$ preoperatively to $81.3\% \pm 15\%$, one month after surgery and $83\% \pm 10\%$ one year after surgery. VAS score was significantly reduced in all patients from 7.08 ± 2

preoperatively to 3.35 ± 1.5 at the time of latest evaluation. One patient with preoperatively ASIA D and 2 patients with ASIA C grade improved postoperatively to ASIA E. The one-year survival rate from the date of diagnosis was 85.2% (95% CI, 60.6%

- 96.0%), while the five-year survival rate dropped to 55.4% (29.4% - 75.1%). The one-year survival rate from the date of surgery was 65.9% (95% CI, 38.8% - 83.2%), while the five-year survival rate dropped to 33.5% (95% CI, 11.1% - 58.0%).

DISCUSSION & CONCLUSIONS: Palliative surgical treatment for osteolytic painful vertebral lesions in MM patients was proved to prevent vertebral body collapse and potentially spinal cord injury, restore vertebral body and improve pain and quality of life.

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Chloride Containing Bioactive Glasses

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INTRODUCTION: Bioactive glasses dissolve in body fluids, releasing calcium phosphate and silicate ions and forming apatite like phases. They stimulate new bone formation and exhibit excellent osseointegration.

Previous studies have investigated the incorporation of fluoride in bioactive glasses [1,2]. The chloride ion is significantly larger than the fluoride or oxygen anions and this is likely to strongly influence the glass structure, dissolution behaviour and ability to form apatite like phases. Chloride ions are naturally present in the body and consequently unlike fluoride ions which are present only at low concentrations chloride releasing glasses are likely to be more acceptable.

This study investigates the incorporation of chloride into bioactive glasses on their dissolution behaviour and ability to form apatite like phases in Tris buffer and simulated body fluid (SBF).

METHODS: Glasses were synthesised using a melt quench route by progressively adding CaCl₂ to the starting glass composition B3 given in Table 1.

Table 1. Glass Compositions Synthesised (mole %)

Glass	SiO ₂	CaO	P ₂ O ₅	CaCl ₂
B3	37.27	44.12	6.18	0.0
C3	36.82	47.74	6.11	2.28
D3	36.39	49.63	6.04	3.46
E3	35.4	51.55	5.87	4.6
F3	34.08	52.98	5.65	7.17
G3	32.8	53.61	5.44	10.64
H3	30.29	54.27	5.02	14.02

All the glasses have a fixed network connectivity [3] of 2.08.

The glasses were characterised by XRD and high temperature differential scanning calorimetry (DSC) and their dissolution behaviour in Tris buffer pH=7.4 and SBF and ability to form apatite like phases characterised by Inductively Coupled Plasma Optical Emission spectroscopy, FTIR, XRD and ³¹P MAS-NMR.

RESULTS: All the glasses were largely amorphous by XRD. The glass transition temperature determined by DSC decreased markedly with CaCl₂ content reducing by over 250°C The glasses dissolved more quickly on incorporating CaCl₂.

The glasses showed evidence of apatite like phase formation in Tris buffer after immersion for as little as 3 hours. Apatite formation took slightly longer in SBF. There was some evidence that these glasses were so reactive that they reacted with atmospheric water.

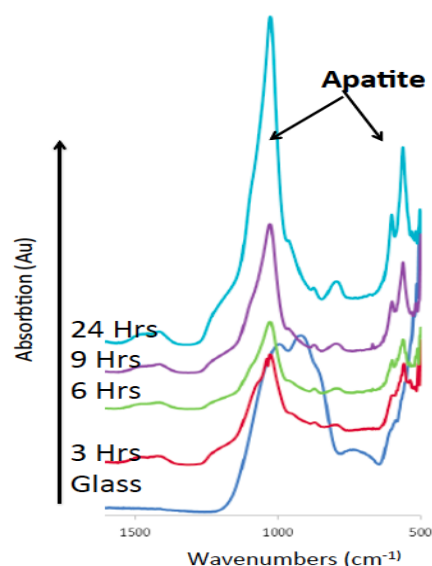


Figure 1 FTIR Spectra of Glass C3 after immersion in Tris buffer.

DISCUSSION & CONCLUSIONS: Novel bioactive glasses were synthesised containing chloride. The incorporation of CaCl₂ resulted in less crosslinked and more reactive glasses that dissolved more rapidly and formed apatite like phases very quickly.

Further studies are required to assess the in vivo behaviour of these glasses.

These novel glasses may be suitable for bone substitutes tissue engineered scaffolds and remineralising toothpastes.

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In-vitro to in-vivo developmental pathway of a novel injectable bioactive glass cement for bone grafting in dentistry

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INTRODUCTION: Currently the only synthetic option available for bone grafting in dentistry is granular calcium phosphate ceramic materials. These materials have been used successfully offering an osteoconductive and somewhat resorbable bone substitute material. Their disadvantages however are susceptibility to deformation & migration, poor handling properties and have poor *in-vivo* performance.

In dentistry bone graft materials are used for a number of procedures including guided bone regeneration (GBR) and alveolar socket filling (ASF). The problems of current bone substitute materials used in dentistry are primarily that they do not set *in-vivo* and the difficulty of implantation cause by their granular nature. It has been the aim of this project to overcome these challenges by developing a novel injectable, *in-vivo* setting calcium phosphate cement; with *in-vivo* porosity that can be mixed and injected through an injection delivery device.

METHODS: Cements were developed and optimised, as outlined previously¹. The bioactive glass and $\text{Ca}(\text{H}_2\text{PO}_4)_2$ powders were mixed together in their specific ratios. The cement powder was then mixed with a 2.5% Na_2HPO_4 solution. Cement compositions were chosen based upon working and setting times, compressive strength, and cements phase formed. A six and twelve week femoral ovine study was conducted using four hand-mixed cement compositions, mixed on a glass slab. The *in-vivo* specimens were analyzed using X-ray microtomography, SEM, and confocal microscopy. Cement compositions were then developed for dental procedures. This involved incorporation into the Medmix Bone-Cement Delivery System (P-System). Liquid to powder ratios of the various cement compositions were to allow mixing and subsequent injection through the syringe.

RESULTS: Initial observation of *in-vivo* bone sections indicated no inflammatory reaction or fibrosis. Bone formation was observed in the cement at 6 and 12 weeks. Bone formation was seen in the 6 week samples predominantly adjacent to the outer surface of the biomaterial.

At 12 weeks however, bone was observed more uniformly distributed within the implant with areas of osteogenesis observed. At six weeks there was a thin layer of bone covering the surface of the implant whereas after twelve weeks the new bone covering the implant was relatively thick.

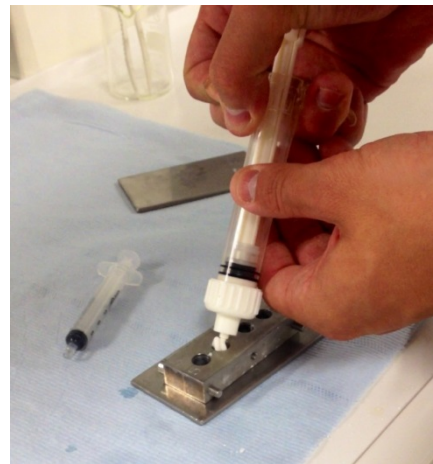


Fig 1 Novel Bioactive glass cement being injected through the Medmix P Cement Mixing System

All cement compositions could be injected using the delivery syringe. The use of the syringe did not impact either the compressive strength or the setting times when compared against mixing the cement on a glass slab.

DISCUSSION & CONCLUSIONS: The novel bioactive glass-based cement system overcomes the disadvantages associated with bone graft materials in dentistry. The Medmix Bone-Cement Delivery System (P-System) enables a more efficient controllable and precise delivery. The bioactive glass-based cement was able to be premixed homogeneously in the delivery device and injected through a curved cannula with an inner diameter of 2.5mm into the field of interest. The results of the ovine indicated that all the cement samples studied are osteoconductive and are able to osseointegrate with host bone.

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Repair of bone defect using zinc-doped bioactive glass: an experimental study in an irradiated rat model

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INTRODUCTION: The Zinc (Zn) is an essential mineral that is a component of more than 200 enzymes and is known as to be necessary for normal collagen synthesis and mineralization of bone (1). Bioactivity of bioactive glass (BG) has been shown to be dependent on its chemical composition and structure. In order to improve the bioactivity and other properties of the material, several elements such as Zn, have been incorporated into BG (2). The purpose of this study was to evaluate the importance of incorporating Zn into BG on bone mineralization.

METHODS: A drill-hole was done in the femoral condyle of female rats and then filled with 10 mg BG or Zn-BG. Surgical interventions were performed under general anesthesia in aseptic conditions. In *experiment 1* (Tr1Gy (perfect 1Gy group), BG1Gy (bioglass pure 1 Gy group), Zn-BG1Gy (zinc doped bioactive glass 1 Gy group)), 30 days after surgery, rats were exposed to 1 Gy, and after an additional 10 days they were euthanized and tissues were harvested. In *experiment 2* (Tr2 Gy (perfect group 2 Gy), BG2Gy (bioglass pure 2Gy group), Zn-BG 2Gy (zinc doped bioactive bioglass 2 Gy)), 30 days later they were exposed to 2 Gy and after an additional 3 days they were euthanized and tissues were harvested.

Femoral condyles were placed in 25 ml tubes with 2 ml of added nitric acid. One milliliter of 30% H₂O₂ was placed in the tube after 10 min. The volume of the mixture was made up to 500 ml with distilled water. Standard solutions of Ca and P were used to prepare the working standard solution, and a blank solution. The element concentrations were detected using inductively coupled plasma optical emission spectrometry ICP-OES. Biochemical parameters were detected by kits.

RESULTS: ICP-OES and biochemical parameters showed an increase of Ca, Ca/P ratio and alkaline phosphatase in Zn-BG groups, this explain bone mineralisation in bone.

DISCUSSION & CONCLUSION: Zinc has been known to encourage attachment, proliferation of osteoblast and increase ALP expression. The enzymes responsible for laying down the bone callus were activated by Zn, and Osteon and Osteoid structures are known to have a high Zn content (3). So zinc has an important role for the mineralization of bone.

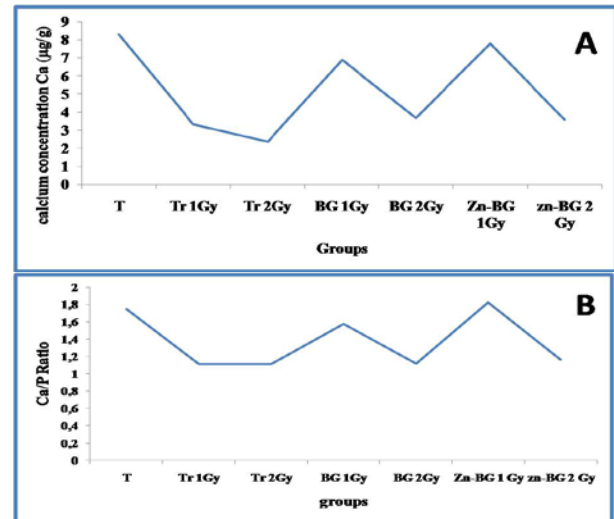


Fig.1. Distribution of Ca (A), Ca/P (B), in newly-formed bone of irradiated female Wistar rats implanted with bioglass (BG) and zinc-doped bioglass (BG-Zn).

Table 1: Biochemical parameters in the serum of irradiated female wistar rats, implanted with bioglass (BG) and zinc-doped bioactive glass (Zn-BG).

	T	Tr2Gy	Zn BG2Gy	BG2Gy	Tr1Gy	Zn BG1Gy	BG1Gy
ALP (µkat/l)	209.5 ± 1.8	175.25 ± 2.27*	205 ± 2.45#	203 ± 3.5#	200.5 ± 3.55*#	208.5 ± 3.76*#@	206.5 ± 5.23*#@
P (mmol/l)	2.52 ± 0.15*	1.9 ± 0.03*	2.21 ± 0.1*	1.9 ± 0.12*	1.51 ± 0.04*	1.85 ± 0.028*	1.51 ± 0.049*
Ca (mmol/l)	2.73 ± 0.077	1.61 ± 0.14*	2.13 ± 0.0047**	2.23 ± 0.06*	2.36 ± 0.62*#	2.59 ± 0.06*	2.38 ± 0.016*

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Reversible dentine demineralisation

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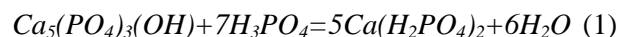
² *SIMAD, IUT Paul Sabatier LU50, Toulouse III, Fr.*

INTRODUCTION: The burr used to remove carious dentine leaves a smear layer on treated dentine. 33% H₃PO₄ is commonly used to remove debris and dissolve the smear layer prior to infiltration of monomers intended to stabilize demineralized collagen when polymerized. However, water is used to remove the dissolved products and remains in the demineralized zone of dentine, requiring a hydrophilic monomer to enable monomer infiltration in the water expanded collagen. Long term clinical observations reveal that the weak link in the aesthetic restorations built with polymers, glass-ionomer resins or composite materials containing organic adhesives is the sound dentine to demineralized dentine interface, causing eventual loss of restoration [1]. Several attempts were made to improve this situation by applying ethanol rinsing prior to adhesive application, or by integrating biologically active ingredients in the infiltration medium to prevent metal matrix proteinase (MMP enzyme) or bacterial degradation of the unprotected water laden collagen, but these solutions do not fill the demineralized zones completely with biologically inert and hydrophobic material. We report on the possibility of dentine remineralisation following acid removal of the smear layer

METHODS: Dentine powder was made by low temperature grinding of healthy molars extracted for prophylactic reasons. The outer enamel layer was removed prior to grinding [2].

33% Phosphoric acid was used to treat the dentine powder, but the dissolution products were not rinsed away, but left to remain in the final material to enable chemical analysis of reaction products. The acid treated dentine powder was investigated by IR spectroscopy, 31P NMR, thermal analysis (TGA and DSC) and XRD. To evaluate the possible reversibility of the demineralization, the treated dentine was reacted with calcium hydroxide and followed by chemical analysis during several weeks during aging in an oven thermo stated at 40°C and 80% humidity.

RESULTS: As expected (see Fig.1), H₃PO₄ reacted with the hydroxyapatite contained in dentine to produce monocalcium phosphate according to equation (1):



Following reaction with calcium hydroxide, the acid calcium phosphate gradually converted back to a hydroxyapatite, but only slowly (3 to 4 weeks) in a carbonated form.

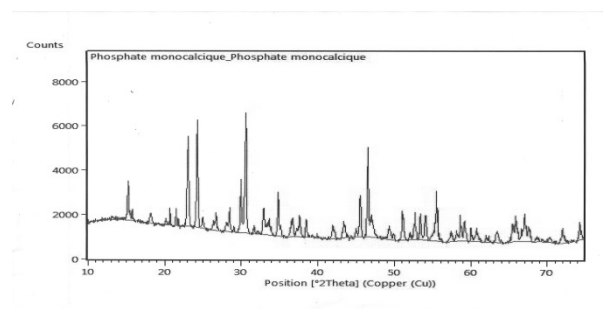


Fig. 1: X-ray diffraction pattern of the calcium phosphate produced by acid reaction with dentine.

DISCUSSION & CONCLUSIONS: The remineralisation of demineralized dentine is thought to be possible by biomimetic deposition of calcium from physiological serum, but this should be a very slow process inside living teeth. Dentists use calcium hydroxide to treat the inside edge of teeth where a small proportion of dentine remains after removing carious infections. Our results demonstrate that the hydrated collagen matrix resulting from acid demineralization can be theoretically re-mineralized in vitro by calcium hydroxide to replenish the space left void by hydroxyapatite removal during smear layer cleaning and dentine etching.

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Development of an injectable extracellular matrix for regenerative medicine by silanization of a cellulose derivative in an ionic liquid medium.

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INTRODUCTION: Hydroxypropylmethylcellulose (HPMC) is a biocompatible polymer and a good candidate to make injectable hydrogels. After chemical grafting of silanolate groups under basic conditions, the decrease of pH promotes a self-crosslinked network. Innovative Extra Cellular Matrices (ECMs) are obtained thanks to these particular chemical groups which induce siloxane covalent bond between HPMC chains. In regenerative medicine, these materials are currently evaluated in animal models for tissue engineering by combining autologous stem cells, and thus producing hybrid synthetic biologic grafts. Our group focuses in a new process of silanization in ionic liquid (IL) media. These media used as solvents have highlighted a real improvement on the number of linked silanolate onto the polysaccharide.

METHODS: Working in IL media with cellulose and its derivatives allow to obtain homogeneous reaction conditions, and thus, a better access to the functions. A previous work [1] on silanization of HPMC (Colorcon®, E4M, $\overline{Mw} \approx 370$ kg/mol) in alkaline conditions has given high functionalization rate. Nevertheless, it appeared to be harmful for the polysaccharide. Our aims today are: (i) to found reactive conditions friendly to the HPMC, (ii) to obtain controlled functionalization rates. The IL 1-allyl-2,3-dimethylimidazolium dicyanamide (AMMIM DCA) was chosen for its good ability to solubilise HPMC and for its liquid nature at room temperature. Sodium hydroxide was chosen as a positive control of the silanisation with (3-glycidopropyl)trimethoxysilane (GPTMS). We evaluated the influence on the resulting polysaccharide of major parameters involved in the reaction conditions such as: the base (organic vs. inorganic), the temperature and the presence of water (not detailed). Molecular weights were determined using a high performance size exclusion chromatography (HPSEC, Shimadzu) combined with a multi-angle laser light scattering detector (MALS, Wyatt technology). Analyses of the silicon content were done by

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). In a typical silanization

experiment, AMMIM DCA (3.25g) is weighted in a 10 mL round bottom flask, inerted with nitrogen and heated at 60°C. HPMC (100mg, 1eq.) is then solubilized within 1h at 60°C. The basic activating agent (NaOH_(s) or (l), DBU, DBN, DABCO, *t*-BuOK, 1eq.) is introduced and the medium is stirred at 60°C for 30 min. Then, GPTMS (1eq.) is added slowly *via* a syringe and the reaction is carried out for 2h. The resulting mixture is then dialyzed and freeze-dried.

RESULTS:

Table 1. Molecular Weights and weight percentage silicon in the products after silanization procedure with different bases.

	NaOH _(s)	NaOH _(l)	DABCO	DBN	DBU	<i>t</i> -BuOK
\overline{Mw} (kg/mol)	290	256	294	400	365	332
Si wt%	2.55	0.74	0.04	0.18	1.74	0.55

These new reaction conditions have significantly improved the preservation of the HPMC chains in IL media. The molecular weights of the products were sensibly similar to that of the starting material, excepted in case of aqueous sodium hydroxide base. Moreover, high functionalization rates (theoretical maximum = 8 wt%) were obtained with NaOH_(s) and the DBU.

DISCUSSION & CONCLUSIONS: Organic bases seem to be less degrading against HPMC chains and thus more adapted for further experiments. It was also observed that the presence of water induced a depolymerization in IL media, explaining the lower \overline{Mw} with aqueous sodium hydroxide. This improved process should permit to establish a methodology allowing control on the functionalization rates, respectful of the chains and potentially allowing transposition to other polysaccharides.

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ACKNOWLEDGEMENTS: ANR Ionibioigel and « Région Pays de La Loire ».

Development of a method for testing bovine caudal discs for biomechanical assessment of novel nucleus replacement therapies.

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INTRODUCTION: More than 75% of cases of low back pain are associated with degeneration of the intervertebral discs, it is reported that 97% of individuals over the age of 50 years display evidence of disc degeneration¹. Nucleus replacement potentially offers a minimally invasive treatment for early stage disc degeneration, but there are limited methods available for testing these treatments in the laboratory. The aim of the present study is to develop standardized in-vitro testing methodologies to examine the biomechanical performance of nucleus replacement procedures.

METHODS: Fresh bovine caudal discs were excised and immediately frozen at -80°C. Whilst frozen, specimens were dissected such that 15 mm of flat, parallel bone surfaces either side of the disc remained attached. This was achieved using a custom made cutting guide in combination with high resolution CT imaging at a voxel size of 246µm (Xtreme CT, Scanco, Switzerland). A method was developed to enable the nucleus to be extracted from a disc with minimal damage to the annulus fibrosus. A 9.5mm hole was drilled through the center of the superior bone surface of the disc. Drilling was ceased when the superior bony endplate was breached. A biopsy punch (8mm diameter, Steifel, USA), ring curette (4mm, Steifel, USA) and a pair of fine tweezers were used to carefully remove nucleus material, which was weighed immediately using a lab balance (Oheus Pioneer, Switzerland). An oversized allograft (with endplate) of predetermined length (10mm in diameter) was obtained from a donor disc to create a push-fit interface between the hole created and the allograft. This technique created a void within the disc for subsequent testing and nucleus replacement injection through a fine gauge needle (30G). In the present study a nucleus augmentation material simulant was used, with similar viscosity to the materials currently in development. This was used as an indicator to examine signs of leakage within the intervertebral disc.

RESULTS: The method of generating a standardised nucleus removal model to allow testing of novel nucleus replacement materials appears to be successful. The technique used, enabled controlled removal of nucleus material whilst maintaining the

integrity of the annulus fibrosus. MicroCT and photographic images of the replaced bone plug confirm that a good seal between the vertebra and the bone plug was created, minimizing leakage and maintaining endplate morphology (Figure 1).

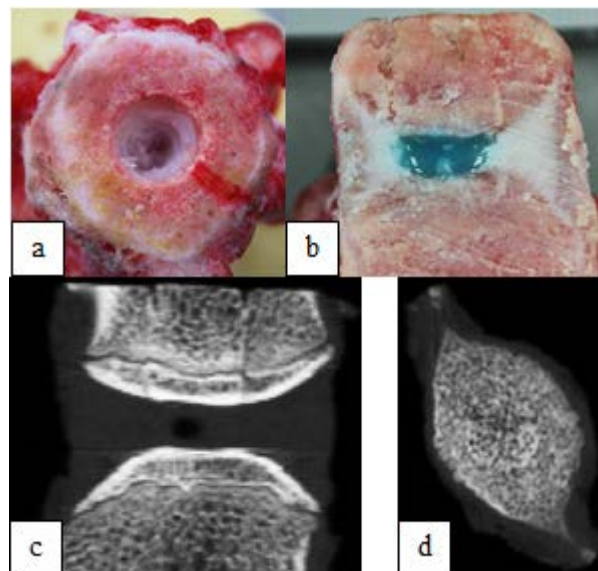


Fig. 1: a. Transverse plane view of specimen following controlled nucleus removal, b. Injected peptide simulant (for image purposes), c. Sagittal CT slice showing placement of bone plug, d. Interface between vertebra and bone plug.

DISCUSSION & CONCLUSIONS: Using the methods described further studies will use a combination of static and dynamic biomechanical testing to determine if novel minimally invasive nucleus replacement therapies, currently in development at the University of Leeds, can be utilised to restore mechanical properties of intervertebral discs following nucleus removal.

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ACKNOWLEDGEMENTS: Funding provided by the EPSRC, the Wellcome trust and the National institute for health research as part of a collaboration with the Leeds Musculoskeletal research unit (LMBRU).

IN VITRO EVALUATION OF OSTEOGENIC AND ANTIBACTERIAL ACTIVITY OF A SYNTHETIC BONE SUBSTITUTE MODIFIED WITH COLLAGEN-HYALURONIC ACID-VANCOMYCIN COMPLEXES

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INTRODUCTION: Periprosthetic infection (PPI) is a devastating consequence of implant insertion involving 1%–5% of procedures, further elevated in trauma patients. Strategies to PPI prevention involve either increasing the rate of new bone formation or the release of antibiotics such as vancomycin (Va). Modern surface-engineering approaches allow to combine these strategies: we developed an innovative synthetic phosphate-based ceramic bone substitute, surface modified with a coating composed of collagen (Col), hyaluronic acid (Hya) and vancomycin (Va) complexes. The aim of this work is to test the pro-osteogenic and antibacterial activity of Col-Hya-Va treated ceramic granules, by *in vitro* assays involving measurement of gene expression of bone and inflammatory cells, antibiotic elution rate and zone of inhibition of bacterial growth.

METHODS: Phosphate-based ceramic granules (dimensional range of 300 μm – 2 mm) were subjected to a surface coating treatment with collagen, hyaluronic acid and vancomycin complexes. This surface process generates a coating with a thickness of hundreds of nanometers. The granules were then characterized by FT-IR, scanning electron microscopy (SEM) and the profile of antibiotic release was evaluated through HPLC and UV-visible spectroscopy. Biological properties were tested on inflammatory (J774.A1) and bone cells (SaOs-2): morphology and gene expression (RT-PCR) studies at different timepoints were performed. The bactericidal effect was evaluated by agar germ test with a *Staphylococcus Epidermidis* strain.

RESULTS: Physico-chemical studies demonstrated that the production process was reproducible and that granules had an identical degree of porosity and similar mechanical and degradation properties. The evaluation of vancomycin release indicated that more than 70% of vancomycin was released during the first 3 days. The agar germ test (figure 1) confirmed that the entrapped antibiotic is able to preserve its bactericidal properties, by inhibiting the growth and proliferation of *S. Epidermidis*.

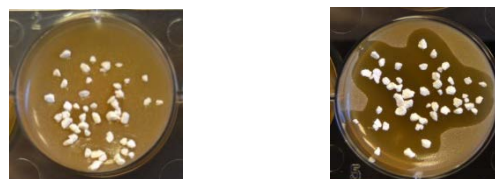


Fig. 1: Anti-bacterial effect of surface modified granules (left-control; right-test sample)

In vitro biological studies (table 1) demonstrated that collagen-hyaluronic acid complexes on the surface of the granules stimulate the expression of pro-osteogenic genes (such as those for ALP, BSP and BMP-2) in bone cells while decrease inflammatory response in macrophage cells (expression of IL-1 β , IL-6 and MCP-1).

Table 1: Gene expression of bone cells and inflammatory cells (+ or – compared to untreated granules)

Bone cells	Col-Hya-Va	Inflammatory cells	Col-Hya-Va
ALP	++	MCP-1	---
BSP	+	IL-1 β	---
BMP-2	+	IL-6	---

DISCUSSION & CONCLUSIONS: *In vitro* results show that this surface treatment is able to add biochemical stimulation and bactericidal functionality to the mechanical characteristics of the synthetic ceramic bone substitute. Even if the encouraging *in vitro* results must be confirmed by further *in vivo* tests, this study demonstrates that surface engineering applied to synthetic bone substitutes could play an important role in the development of innovative materials of clinical interest.

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Surface chemistry and effects on bone regeneration and inflammation of a novel biomimetic synthetic bone filler

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INTRODUCTION: The lack of dentoalveolar bone could often disallow implant therapy by compromising primary stability. Thus, replacement of bone loss or reconstruction of bone defects is still a clinical challenge. Many different bone substitute such as autografts, allografts, xenografts, synthetic biomaterials and osteoactive agents have been proposed. Synergoss® (Nobil Bio Ricerche srl, Italy) is a recently developed synthetic bone filler that exploits biomolecular surface engineering to deliver directly to the filler-implant interface the signaling properties of type I collagen. The aim of this work is to test physico-chemical and biological (*in vitro* and *in vivo*) properties of this ceramic bone substitute compared to its counterpart without collagen surface layer and to the widely used Bio-Oss (Geistlich).

METHODS: Synergoss® (SO) was prepared by mixing nano-hydroxyapatite, β -tricalcium phosphate and a polysaccharide-based porogen. After sintering and sieving, ceramic granules (dimensional range of 300 μ m – 1 mm) were subjected to a surface coating treatment with collagen type I. The granules were then characterized by FT-IR, scanning electron microscopy (SEM), XPS, BET and IGC. Biological properties were tested *in vitro* on inflammatory (J774.A1) and mesenchymal stem cells (hMSCs): morphology and gene expression (RT-PCR) studies at different timepoints were performed. *In vivo* evaluations were performed in a rabbit model as recommended by the international rules UNI EN ISO 10993-6: 2009 to obtain information about material performance in terms of histocompatibility and osteointegrative capabilities (Rizzoli Orthopaedic Institute).

RESULTS: Surface analysis confirmed that the ceramic phosphate granules present a collagen nanolayer to the surrounding environment. Cell cultures tests showed that, in agreement with literature reports, surface-immobilized collagen molecular cues can stimulate progression along the osteogenic pathway of undifferentiated human mesenchymal cells. Moreover SO elicited a strong decrease of the

expression of the main pro-inflammatory cytokines compared to the collagen free control and Bio-Oss (Table 1).

Table 1: Gene expression of hMSCs and inflammatory cells (+ or – compared to untreated granules)

hMSC	Synergoss®	Inflammatory cells	Synergoss®
ALP	++	MCP-1	---
BSP	+	IL-1 β	---
BMP-2	+	IL-6	---

Finally, *in vivo* test in a rabbit model of critical bone defect shows statistically significant increase of bone volume and mineral apposition rate between the biomimetic bone filler and collagen-free control (Figure 1).

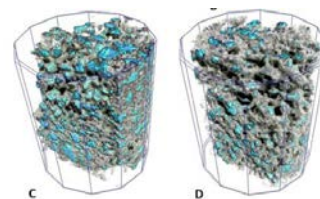


Fig. 1: 3D reconstruction of the Volume of Interest (VOI) selected to perform the microtomographic investigations of SO (C) and SO- (D) materials

DISCUSSION & CONCLUSIONS: All together, obtained data confirm that biomolecular surface engineering can upgrade the properties of implant device, by promoting more specific and targeted implant-host cells interactions.

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Generating *Nucleus pulposus*-like cells from human Adipose Stromal cells: a first step towards the regeneration of intervertebral disc

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INTRODUCTION: Intervertebral disc (IVD) degeneration is one of the major causes of low back pain. It is now well-established that the *Nucleus pulposus* (NP) is prematurely affected by degenerative events, mainly starting with the apoptotic death of the NP resident mature cells namely the nucleopulpyocytes. In this context, repopulating a damaged NP with functional regenerative cells seems to be a promising approach for long term regeneration of IVD. Since Growth Differentiation Factor 5 (GDF5) plays a crucial role in IVD anabolism and biological activity of nucleopulpyocytes [1], we questioned whether GDF5 may be an efficient tool to drive the nucleopulpyogenic differentiation of human Adipose Stromal Cells (hASC). In this context, the aim of the present work was to investigate the effects of supplementing a chondrogenic medium with GDF5 on the *in vitro* production of functional nucleopulpyocytes from hASC.

METHODS: Human ASC have been cultivated in pellets (500.000 cells/pellet) for 28 days in the presence of low oxygen tension (5%) with a chondrogenic medium enriched or not with GDF5 (100ng/ml). The nucleopulpyogenic differentiation of hASC has been evaluated by determining the expression levels of ACAN, COL2A1, PAX1, OVOS2 and CD24 transcripts by RT-qPCR. The levels of the corresponding proteins in hASC pellets have been assessed by immunohistochemistry analyses. The specificity of the hASC commitment has been determined by analysing the expression of osteogenic-, adipogenic- and chondrogenic-related markers by Taqman Low Density Array. Furthermore, hASC were cultivated in the presence of the GDF5-enriched chondrogenic medium with or without SB505124 (5µM) or dorsomorphin (5µM) to decipher the involvement of Smad proteins.

RESULTS: Our RT-qPCR and immunohistochemistry data showed that hASC cultivated in the presence of a GDF5-enriched chondrogenic medium expressed nucleopulpyocyte markers (ACAN, COL2A1, PAX1, OVOS2 and CD24) at the transcript and protein levels. A time course experiment demonstrated that hASC express nucleopulpyocyte markers as early as 21 days of culture. Furthermore, the osteogenic-, adipogenic- and chondrogenic-related markers remain at a barely detectable level of expression. Concerning the role of Smad pathways during the nucleopulpyogenic differentiation of hASC, our western blot data demonstrated that SB505124 and dorsomorphin respectively inhibit the phosphorylation of Smad2/3 and Smad1/5/8 and thus respectively block the TGF-β and GDF5 canonical pathways. Whereas SB505124 was found to prevent the nucleopulpyogenic differentiation of hASC, dorsomorphin did not significantly affect this differentiation process.

DISCUSSION & CONCLUSIONS: Our data demonstrate that human ASC cultivated in the presence of a GDF5-enriched chondrogenic medium can be committed towards the nucleopulpyocyte lineage. This commitment seems to be reproducible, robust and specific. Our results also suggest that the canonical TGF-β pathway (Smad2/3) plays a pivotal role for the nucleopulpyogenic commitment of hASC. Whether the *in vitro* generation of functional nucleopulpyocytes may help us design regenerative medicine strategies remains to be further evaluated in suitable animal models of disc degeneration.

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Strontium substituted C₄P bone cement with enhanced radiopacity and mechanical properties

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INTRODUCTION: Strontium (Sr) has found to hinder osteoclast, promote osteoblast and aid in osseous collagen formation besides being also antimicrobial¹. Sr containing acrylic cements and α/β -TCP based calcium phosphate cements (CPC) has been extensively studied and Sr has found enhance radiopacity in these cements. Recent attention on tetracalcium phosphate (TTCP-C₄P) based apatitic cements has resulted in its usage in clinics worldwide. In this work we have studied the 5 and 10% Sr substituted TTCP based cements.

METHODS: Pure TTCP was prepared by heating equimolar amount of calcium carbonate, (CaCO₃) and dicalcium phosphate anhydrous (CaHPO₄) to 1500°C for 6 h followed by rapid quenching. Strontium carbonate (SrCO₃) was added to the precursor mixture replacing CaCO₃ at 5 and 10 at.% of Sr to that of Ca to synthesize Sr substituted TTCPs by the same procedure. CPC's made of powder containing pure TTCP, 5 and 10% Sr substituted TTCP were named as T₄CPC, 5SrT₄CPC and 10SrT₄CPC respectively. 1M disodium hydrogen phosphate containing 15% citric acid was used as the liquid. CPC powder and liquid are mixed in an L/P ratio of 0.5ml/g. The paste was packed into a mould and allowed to harden. Setting time and compression strength (CS) was measured. Radiopacity was measured by medical X-ray apparatus and compared with pure Al of various thicknesses (ISO 6876:2001). Cell viability (MTT) assay, cell attachment and proliferation studies were done using rat skeletal muscle cells.

Table 1: List of various parameter of CPC

Samples	Setting time (minutes)		CS (MPa)	CDHA (%)	Cell viability (%)
	Initial	Final			
T ₄ CPC	9±1.3	16±3	10.4±3.1	29±7	94±0.5
5SrT ₄ CPC	6±2.4	13±2.5	18.6±5.5	61±8	93±1.4
10SrT ₄ CPC	10±1.3	16±3.2	14.3±4.4	52±6	90±3.5

RESULTS: X-ray diffraction (XRD) pattern of 5 and 10% Sr-substituted TTCP was found to be similar to that of pure TTCP (JCPDS no. 25-1137). The setting

time, CS, cell viability and percentage of calcium-deficient hydroxyapatite (CDHA) formed at 24h (calculated from XRD) are listed in Table 1. Radiopacity increased with increasing of Sr²⁺ concentration as shown in Fig1.

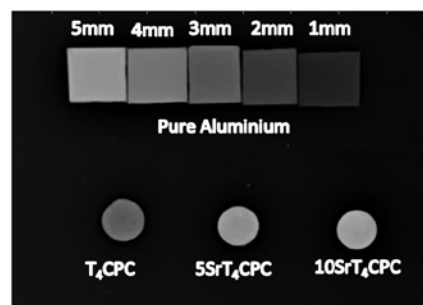


Fig. 1: Radiopacity of Sr substituted CPCs.

DISCUSSION & CONCLUSIONS: Substitution of Sr²⁺ ion into the lattice of TTCP has resulted in expansion of the cell parameters due to larger radius of Sr²⁺ ion than that of Ca²⁺ ion. Setting time was found to be shorter and CS at 24h was found to higher for 5SrT₄CPC. The increased defect concentration with the Sr²⁺ ion substitution in the lattice may have led to the accelerated hydration to form CDHA and this in turn contributed to increased CS of the 5SrT₄CPC. However, 10SrT₄CPC had comparatively slow setting time and low CS at 24h which could be explained due to the inhibition effect of dissolved Sr²⁺ during the early hydration to form CDHA². Increased radiopacity with the increasing Sr content is due to its higher atomic number and weight than that of Ca resulting in increased absorption of X-ray energy³. Cell viability was above 90% for all the samples which proves cytocompatibility of Sr¹. These findings shows that Sr²⁺ substituted C₄P cements could serve as a better bone graft material with potential clinical applications.

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Association of a biomimetic cement with sodium fusidate as a drug delivery bone substitute material

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INTRODUCTION: Calcium phosphate cements have great potential as carriers for controlled release of drugs in bone tissue due to their composition close to bone mineral, excellent bioactivity and possible use as injectable and degradable grafting materials [1]. The objective of this work is to study the injectability and the release property of a biomimetic cement loaded with sodium fusidate (SF), a soluble salt of fusidic acid, an antibiotic with a broad antibacterial spectrum and a good bone penetration.

METHODS: The cement paste was obtained by the homogenous mixing of solid phase (powder mixture of vaterite, CaCO₃, and dicalcium phosphate dihydrate, DCPD, with a weight ratio of 1:1), with an appropriate amount of liquid phase (deionised water), at a liquid-to-powder ratio (L/P) of 0.7 and 0.8 mL/g [2]. An aqueous solution of SF was used as liquid phase to prepare the antibiotic-loaded cements including 3% or 9% of antibiotic (w/w of the solid phase). After mixing the solid and the liquid phases, the paste was placed in a sealed container saturated with H₂O at 37°C for setting and hardening. The cements prepared were characterised by X-ray diffraction, FTIR spectroscopy and scanning electron microscopy. The setting time was determined using the Gillmore needle method. The injectability of the cement was measured using a texture analyser equipped with a syringe system. The release of the antibiotic from the cements was investigated for five weeks in 1 liter of 0.9% w/w NaCl solution at pH = 7.4 using a dissolutest apparatus according to the European pharmacopoeia.

RESULTS: The results indicated that the incorporation of sodium fusidate in the cement reduced the setting time (Table 1), while increasing the injectability of the cements and this effect was dose dependent. In addition to the effect of the antibiotic, cements prepared with the highest L/P ratio were more injectable but exhibited a higher setting time. The drug-loaded cements showed a sustained release, with a release rate of drug which diminished when L/P decreased. For cement containing 9% of antibiotic, the time necessary to release 20% of drug (t_{20%}) was 27 days and 12 days for L/P = 0.7 and L/P = 0.8, respectively.

Table 1: Setting time of the cements.

Cement	Setting time (min)	
	L/P= 0.7	L/P= 0.8
0% SF	75 ± 2	100 ± 4
3% SF	65 ± 3	85 ± 3

DISCUSSION & CONCLUSIONS: The association of SF with the cement increased its injectability but decreased its setting time. The latter observation can be correlated with the study of the setting chemical reaction rate by FTIR spectroscopy which confirmed that the introduction of SF accelerated the dissolution of DCPD and the formation of the biomimetic apatite. Drug release test has shown that SF release rate was dependent on cement microstructure. Indeed, the increase of L/P ratio induced an increase of cement microporosity enhancing diffusion phenomenon and therefore drug release. The possibility to control cement parameters and properties makes this biomimetic cement a promising candidate especially for the prevention of bone implant-associated infections.

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Fresh traumatic lumbar fractures treated with transpedicular vertebral augmentation and pedicle screw fixation. A comparison of two devices and two bone cements

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INTRODUCTION: This retrospective study compares the efficacy and safety of Balloon Kyphoplasty (BK) with calcium phosphate (Group A) versus KIVA implant with PMMA (Group B) reinforced with three-vertebrae pedicle screw construct for A₂ & A₃ single fresh non-osteoporotic lumbar (L₁-L₄) fractures in 38 consecutive age and diagnosis matched patient populations.

The development of transpedicular, inflatable balloons (BK), implantable stents or other reinforcement devices¹ combined with injectable PMMA and bone cement substitutes has, in the recent few years, made possible to perform a sufficient and safe anterior vertebral column reconstruction through a percutaneous transpedicular approach, thus reducing the surgical and morbidity complications associated with a major anterior reconstruction surgery.

However, despite the advent of BK, the occurrence of extracanal, intracanal PMMA leakage remains a significant however potential disastrous complication with rates ranging from 7% to 10%².

The null hypotheses of this retrospective comparative controlled study were: 1) KIVA implant with low viscosity PMMA can equally with balloon Kyphoplasty (BK) with calcium phosphate restore fractured vertebral body? 2) KIVA implant shows similar extracanal bone cement leakage with BK?

METHODS: Extracanal leakage of either low viscosity PMMA and calcium phosphate (CP) as well as the following roentgenographic parameters: segmental kyphosis (SKA), anterior (AVBhr) and posterior (PVBhr) vertebral body height ratio, spinal canal encroachment (SCE) clearance, and functional outcome measures: VAS and SF-36, were recorded and compared between the two groups. All patients in both groups were followed for a minimum of 26 (Group A) and 25 (Group B) months.

RESULTS: Extracanal CP and PMMA leakage was observed in four (18%) and three (15%) vertebrae/patients of group A and B respectively. Hybrid fixation improved AVBhr, SKA, SCE, but PVBhr only in group B. VAS and SF-36 improved postoperatively in the patients of both groups.

DISCUSSION & CONCLUSIONS: Short segment construct with the novel KIVA implant restored better than BK fractured lumbar vertebral body, but this had no impact in functional outcome. Since there was no leakage difference between PMMA and Calcium phosphate, and no short-term adverse related to PMMA use was observed, we advice the use of PMMA in fresh traumatic lumbar fractures.

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Low-Modulus Resin for vertebroplasty and kyphoplasty

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INTRODUCTION:

Percutaneous vertebroplasty and kyphoplasty have been shown to provide benefit to patients with painful vertebral compression fractures in terms of both pain control and disability resolution.

Patients typically demonstrate rapid and durable pain relief and often regain lost function.

Despite all the benefits, there is a great deal of debate about whether vertebroplasty and kyphoplasty also increase fracture morbidity by either inducing or facilitating subsequent vertebral fractures. The objective of this analysis was to examine the safety and effectiveness of percutaneous balloon kyphoplasty for treatment of osteoporotic vertebral compression fractures with a new generation of bone cement which can be the solution to avoid the potential subsequent fractures

METHODS:

Vertebral augmentation with classical PMMA greatly increases the modulus of the treated vertebra, as its modulus is much higher than the surrounding osteoporotic cancellous bone.

Many studies, as well as evident scientific arguments are sufficient to understand that the use of low modulus bone cement is the solution to be mechanically less aggressive for the bone tissue.

We developed the first low modulus resin which has non aggressive and low mechanical properties.

This resin becomes porous (figure1) in situ; its mechanical properties are thus reduced.

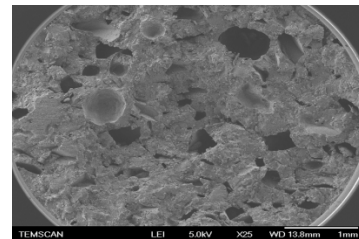
36 patients were treated for recent vertebral compression fractures type A1, A2 by kyphoplasty procedure by transpedicular approach with injection of Resilience®. The clinical follow up was performed during 2 years.

RESULTS:

- Biomechanical tests showed that this resin is less aggressive than conventional bone cement.

- All patients reported relief of their pain, and none of them complained of severe worsening of pain during the follow-up. No subsequent fractures occurred on adjacent levels in all the patients.

Fig. 1: the porous aspect of the Resin.



DISCUSSION & CONCLUSIONS:

For several years, vertebroplasty and kyphoplasty have been successfully used in the treatment of painful osteoporotic compression fractures of the vertebra.

The widely used poly-methyl methacrylate (PMMA) offers a compressive strength which is higher than the trabecular bone.

Accordingly, fractures in the adjacent vertebrae may occur due to the difference in strength from the surrounding vertebrae. Therefore, low-modulus cements may improve the outcome of vertebroplasty and kyphoplasty.

In this study we evaluated the use of a new generation of bone cement, "RESILIENCE[®] Resin", which mechanical properties are close to those of cancellous bone.

The mechanical and clinical studies demonstrate the non-aggressive property and the good clinical outcomes of patients.

This new generation of low modulus bone cement can be used safely, especially on patients with osteoporosis and lower BMD.

Novel implant materials with antibacterial potential for bone regeneration.

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INTRODUCTION: Calcium Phosphates (CaPs) are widely used in the regeneration and reconstruction of bone tissue due to their excellent biocompatibility. They belong to the group of bioactive materials, able to form a chemical bond with natural bone. Materials in the form of easily shapeable cement pastes, based on HA (hydroxyapatite) and CSH (*Calcium Sulfate Hemihydrate*) are very promising bone substitutes. The combination of HA and CSH leads to the fabrication of materials with controlled resorption. Introduction of silver ions into the structure of hydroxyapatite may change its physicochemical and biological properties. Materials with silver are not only able to fill bone defects effectively, but also possess antibacterial properties.

METHODS: Using the wet chemical method silver doped hydroxyapatite (AgHA) was synthesized. Obtained powder was calcined at 800°C (AgHA/c). Calcium sulfate hemihydrate (Acros Organics) was mixed with raw or calcined hydroxyapatite powder. For the cement pastes preparation distilled water and chitosan solution were applied as the liquid phases. Setting times of the cement pastes as well as phase composition, microstructure and mechanical strength of the final materials were examined. Furthermore, their chemical stability, bioactivity and antibacterial character *in vitro* were assessed.

RESULTS: Developed materials set from 6 to 20 minutes. The setting times depended on a type of liquid phase and the kind of hydroxyapatite (AgHA or AgHA/c). Obtained cements after setting and hardening composed of calcium sulfate dihydrate (CSD) and hydroxyapatite. The compressive strength of obtained materials was ~8MPa. Due to the content of CSH, they were characterized by the gradual biodegradation *in vitro*. SEM and EDS studies confirmed the growth of an apatite layer on the surfaces of

tested materials soaked in SBF, what indicates on their bioactive character.

Table 1. Initial composition and setting times of cement pastes.

Cement symbol	Powder (P)	Liquid (L)	L/P [ml/g]	Setting time [min]	
				Initial	Final
A1	AgHA:	Distilled water	0.54	5	9
A2	CSH	Chitosan solution		11	20
A3	AgHA/c:	Distilled water	0.60	3	6
A4	CSH	Chitosan solution		4	8

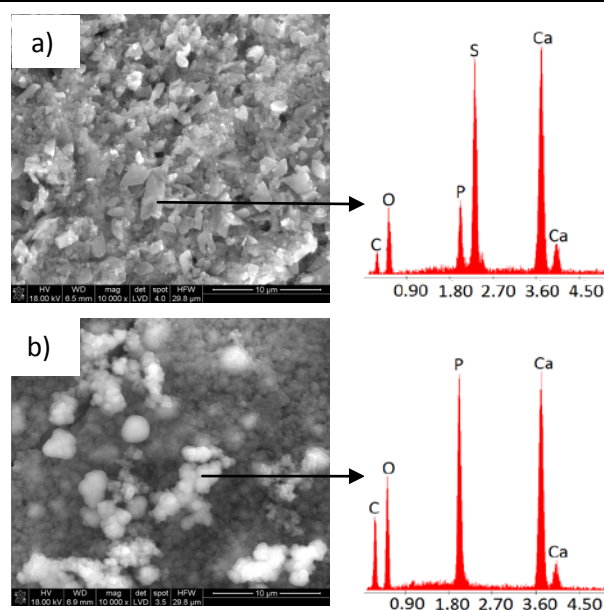


Fig. 1: SEM and EDS images of the cement A4: a) non-incubated, b) incubated in SBF for 7 days.

DISCUSSION & CONCLUSIONS: The new implant materials based on silver doped hydroxyapatite and calcium sulfate hemihydrate were developed. Preliminary biological tests confirmed their bioactivity and antibacterial character.

ACKNOWLEDGEMENTS: The work has been supported by the Polish National Science Centre funds allocated with the decision DEC-2013/09/N/ST8/04171

Injectable ready-to-use calcium phosphate cement

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INTRODUCTION: Calcium phosphate cements (CPCs) are highly valuable materials for filling bone defects and bone augmentation by minimal invasive application. An unpublished survey among European trauma surgeons revealed that improved handling and application properties of CPCs are the most significant prerequisites for furthering the prevalence of respective products in clinical application. In the present study some key features were significantly improved by developing novel injectable ready-to-use calcium phosphate cements based on water-immiscible carrier liquids.

METHODS: Calcium phosphate cements were prepared from 60 wt.% alpha-TCP ($\text{Ca}_3(\text{PO}_4)_2$), 26 wt.% DCPA (CaHPO_4), 10 wt.% calcium carbonate (CaCO_3), and 4 wt.% precipitated hydroxyapatite (HA)¹.

Powder/liquid CPC (pl-CPC) was prepared by mixing the powder composition with 4% Na_2HPO_4 -solution at a liquid to powder ratio L/P = 0.4 ml g⁻¹.

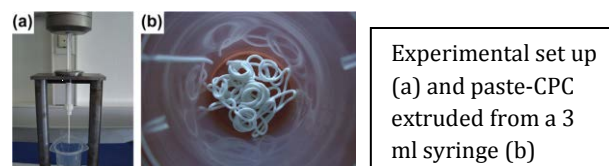
Ready-to-use paste-CPC contained the described CPC powder with addition of 2.5% finely ground K_2HPO_4 in an oil-based suspension (synthetic short chain triglyceride Miglyol 812 with 8-12 C saturated fatty acids) at a powder/oil phase ratio of 85/15. The oil phase contained two surface-active agents, castor oil ethoxylate 35 (Cremophor ELP, BASF, Germany) and hexadecyl-phosphate (Cetyl-phosphate, Amphisol A, Brenntag AG, Germany).

Both cement versions were tested for setting behavior, cohesion, mechanical properties, injectability, and phase transition during the setting process. Except for setting behavior paste-CPC was cured by exposing to 0.9% NaCl solution without active mixing. Setting of paste-CPC was determined after mixing of 2 g cement paste with 0.25 ml 0.9% NaCl solution^{2,3}.

Paste-CPC was additionally tested for storage stability at ambient conditions and in accelerated shelf life testing (55°C, 70% relative humidity). Biocompatibility testing was performed for both CPCs in cell culture and implantation study in rabbits according to ISO 10993 standard.

RESULTS: Paste-CPC showed a reduced setting time, reduced particle release and increased maximum

compressive strength in direct comparison to pl-CPC. For both cement versions slow transition into poorly crystalline HA was demonstrated by XRD at approximately the same speed. Striking differences were observed between both cement versions in terms of injectability. While pl-CPC was difficult to eject from standard syringes and ejection was impossible when a cannula was attached, paste-CPC could be easily extruded from syringes of different sizes with or without attached cannula. Extrusion force for paste-CPC was influenced by syringe type, attached needle, and extrusion speed. In all cases extrusion force reached a plateau and remained constant. During 12 months of storage at ambient conditions a slight increase in extrusion force was observed. No such increase was seen upon accelerated shelf life testing. Paste-CPC was verified to be biocompatible according to ISO 10993 standard, both *in vitro* and *in vivo*.



DISCUSSION & CONCLUSIONS: Using a conventional pl-CPC as basis for a newly developed ready-to-use paste-CPC greatly improved injectability and handling properties by obviating the need for mixing. Moreover relevant cement properties are either comparable or superior to the direct pl-CPC counterpart. Presented data suggest that the transformation of a clinically established CPC formulation into a ready-to-use paste-CPC opens interesting new opportunities for simplified filling of bone defects and advanced augmentation of osteosynthetic hardware.

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Mechanism of formation the thermosensitive chloride chitosan gels for biomedical applications

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INTRODUCTION: Chitosan has been widely applied as a biomaterial for tissue engineering due to its biocompatibility, non-toxicity and biodegradability. Furthermore, injectable thermosensitive chitosan hydrogels are formed by neutralization of acidic chitosan solution using sodium beta-glycerophosphate (β -GP) at physiological pH and temperature. For biomedical applications, thermo-gelling injectable systems with an LCST around or below 37 °C would be ideal, as they would transform from a solution to a gel upon injection into a body cavity. The aim of this study was to investigate and explain the mechanism of thermosensitive gel formation based on literature [1-3] and own research.

METHODS: Chitosan/ β -GP injectable gels were produced. 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. After complete dissolution, samples were stored at 37°C for 24h. The structural properties of hydrogels after conditioning in water were studied. The structural characteristics were analysis of FTIR, XRD and SEM.

RESULTS: The SEM image of chitosan gels is shown in Figure 1.

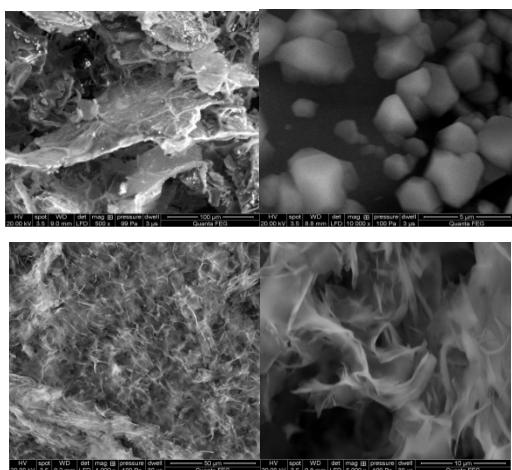


Fig.1: SEM image of chitosan thermosensitive gels before (up) and after (down) conditioning in water.

Based on the experiments the following processes are proposed:

GP dissociates ; this causes a pH increase of the chitosan solution; at low temperatures there is electrostatic interaction between the negatively charged (residues) phosphate molecules from glycerophosphate and positively charged amino groups; GP supports also hydration of chitosan molecules and the interaction of chitosan molecules with water; these processes protect chitosan against immediate separation; between polymer molecules there are physical and hydrogen bonds, however weak; under the influence of temperature electrostatic interaction still exists between the negatively charged (residues) molecules of phosphate from glycerophosphate and positively charged amino groups; water molecules protecting the polymer are removed by glycerin residue and new stronger hydrogen bonds are formed between hydrophobic groups which causes the precipitation of polymer.

CONCLUSIONS AND OUTLOOK: An attempt was made to explain the mechanism of gel formation on the basis of structural studies of thermosensitive chitosan gels conditioned in water. The results obtained confirm the theories of Cho and Chenite rather, but the interpretation suggested by Lavertu is supported by the fact of removal of glycerophosphate from the structure. However, it is disputable whether due to the combination of protons from amino groups with glycerophosphate. Glycerophosphate is released by the combining of protons of the amino groups of chitosan with glycerophosphate, or is removed as a result of cleavage of the sodium.

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CaP nanowires electrodeposition by the template method

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INTRODUCTION: Calcium phosphates (CaP) have attracted much interest for biomedical applications because of their properties (good biocompatibility, osteoconductivity and bioactivity) and their chemical and structural similarities to the bone and teeth mineral component. CaP nanowires are expected to exhibit unique and optimized properties because of their restricted size and high specific surface area and can be used to improve the mechanical properties in bone tissue engineering. Here we report the cathodic electrodeposition of CaP nanowires with high aspect ratio by the template method.

METHODS: Experiments were performed in a three-electrode cell using a Radiometer PGZ301 Potentiostat controlled by Voltmaster4 software. One platinum wire was used as the auxiliary electrode, Ag/AgCl electrode as the reference electrode and gold covered nuclear track-etched polycarbonate membrane Whateman® as template. The thickness and pore diameter quoted by the supplier were 16 μm and 90 nm respectively. The electrolytic aqueous bath is composed of 0,042 Mol $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and 0,025 Mol $\text{NH}_4(\text{H}_2\text{PO}_4)$ [1]. 9 % vol H_2O_2 was added into baths to prevent hydrogen bubbles formation and favour a better calcium phosphate crystal nucleation, more homogeneous deposit and better composition and structure of the synthesised material [2]. The pH was adjusted between 4,2 and 7 by sodium hydroxide (NaOH) addition and a forced convection was set up by using a peristaltic pump. Electrodepositions were performed at 80°C and an applied potential $E = -1600\text{mV}$ during 5 minutes. After electrodeposition, X-Ray diffraction (XRD) analysis of the crystal structures was performed with a Bruker Analytical X-Ray diffractometer ; for scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) analysis, the nanowires were collected by dissolving the membranes by dichloromethane.

RESULTS : X-ray measurements reveal the formation of OCP (octacalcium phosphate - ICDD : 79-423) and/or HAP (hydroxyapatite - ICDD : 9-432). The SEM micrography (Fig 1) shows many nanowires with average length 8 μm and diameter 260 nm. From the EDX analysis, the Ca/P molar ratios of the samples were all between 1.3 and 1.9

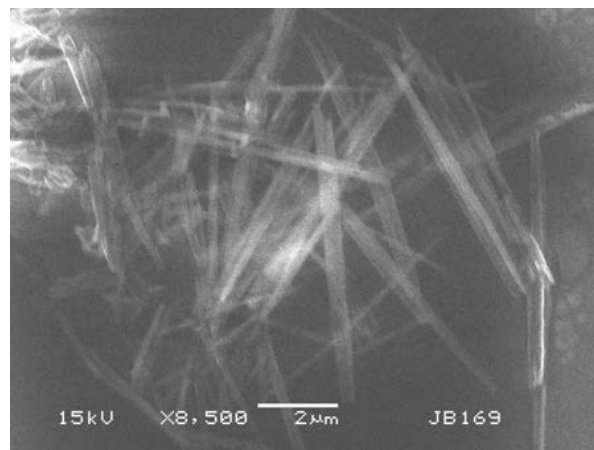


Fig. 1: SEM micrograph of CaP nanowires.

DISCUSSION & CONCLUSIONS :

The XRD spectra and SEM analysis coupled to EDX spectroscopy confirmed the successful synthesis of CaP nanowires with an aspect ratio (length/diameter) of 30, which is higher than the value obtained by other studies [3-4]. These results indicate that CaP growth inside nanoporous membrane could generate longer nanowires which could be used to investigate their specific biological response in bone regeneration.

Complementary analysis will be necessary for validate the nature of components (HA and/or OCP). An amorphous phase (ACP) is probably associated because the respectively Ca/P molar ratios of OCP, HA and ACP are 1.33, 1.67 and 1,2 to 2.2.

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Autologous platelet-rich plasma extra-articular injections for problems around knee joint

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INTRODUCTION: : Platelet-rich plasma therapy (PRP) is based on using a concentrated platelet sample from the same patient and injecting it into the area of an injured tissue.

PRP contains vital substances and cells that create new tissue and repair damage. After taking blood from the patient, the plasma sample is then spun down in a centrifuge to separate the needed healing properties. These constituents can be further selected for a specific area of bone or soft tissue to enhance the body's natural capacity to heal.

METHODS: Thirty-six patients in total were subjected to autologous platelet-rich plasma injection in order to treat various problems around knee joint. In particular, there are twelve patients who suffered from quadriceps muscle injuries, eleven patients suffering from patellar tendinosis, eight patients afflicted with medial or lateral collateral ligament sprain or partial tear, three with iliotibial tract friction syndrome and two with pes anserinus tendinobursitis syndrome. All patients were evaluated with a ten-cm visual analog scale (VAS) to assess pain before platelet-rich plasma injection.

. Blood is drawn from patient's arm and placed in a special processing unit. The blood is then spun in a centrifuge in order to separate platelets, white blood cells and serum from red blood cells. The platelets and white blood cells are then concentrated and collected into a sterile syringe. Some of the blood is used to create an "activator" of the PRP. The skin and soft tissue is anaesthetized with local anaesthetic, followed by injection of both the PRP and activator into the tissue targeted for treatment. Depending on the size of the injured tissue, one or several needles are inserted to optimize the placement of the PRP. Patients returned after 4 weeks for a post injection follow-up. A second injection was given if significant improvement was not obtained by that time. A ten-cm visual analog scale (VAS) was used to assess pain. VAS scores were

collected in four weeks and eight weeks follow-up respectively.

RESULTS: The average age of the patient was 35 years old and gender ratio was 21/15 in favour of men. All the patients were followed up in 4 weeks and 6 months. A second injection was given to 7 patients who showed no significant improvement, and more specifically 3 patients with patellar tendinosis, 2 quadriceps muscle injury, 1 iliotibial tract friction and 1 anserinus tendinobursitis syndrome.

Mean VAS was reduced from 56.5 +/- 20.32 at the time before injection to 38.5 +/- 17 in 4 weeks and 20.57 +/- 16.1 in 8 weeks follow up.

DISCUSSION & CONCLUSIONS: While the clinical benefits of PRP in enhancing the healing of musculoskeletal tissues are still being explored, the substantial amount of basic science data supporting the role of growth factors in enhancing cell migration, cell proliferation, and matrix synthesis has provided a compelling rationale for use of PRP in the treatment and repair of various connective tissue structures, ligament, and muscle injuries. **REFERENCES**

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Platelet-Rich Plasma Therapy for Knee Joint Problems: Review of the Literature, Current Practice and Legal Perspectives in Korea
Yong-Geun Park, MD1, Seung Beom Han, MD2, Sang Jun Song, MD3, Tae Jin Kim, MD3 and Chul-Won Ha, M

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Bioactive glass incorporation in calcium phosphate cement-based injectable bone substitute for improved in vitro biocompatibility and in vivo bone regeneration

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INTRODUCTION: In this paper an injectable bone substitutes (IBS) modified with the addition of bioactive glass powders synthesized via ultrasonic energy-assisted hydrothermal method is reported with improved biocompatibility behaviour. Setting time and compressive strength of the IBS were evaluated and observed to improve with the increase of bioactive glass content. The effects of the bioactive glass concentrations on the material and physical properties of the IBS were also investigated.

METHODS: IBSs were prepared in accordance with the study of Thai et al. [1] by combining: a solid phase made of TTCP, DCPD (Sigma, USA) and CSD (Samchun, South of Korea) and a liquid phase consisted of 2%, 4% HPMC and 10% citric acid. TTCP, 74 wt%, and DPCD, 26 wt%, were mixed in a ball grinder for 8 h in equimolar amounts to obtain the CPC powder. CSD was added in the IBS system at a fixed concentration of 20%. Bioactive glass material was synthesized using ultrasonic energy-assisted hydrothermal method. The powder was prepared by combining $\text{Ca}(\text{NO}_3)_2$, $(\text{NH}_4)_2\text{HPO}_4$ (as 37% SiO_2). The precursors were dissolved in de-ionized water and ultrasonicated for 4 h. The precipitate was washed, filtered, dried and calcined at a temperature of 600 °C. The bioactive glass materials of different concentrations (0%, 10%, 20% and 30%) were mixed to the IBS system at a solid:liquid =1.8:1. Detailed materials characterization and in-vitro and in-vivo experiments were carried out to evaluate the resulting IBS.

RESULTS: In vivo section after 3 months of implantation shown in the images show the collagen deposited across the sample site indicated by the blue stains. The surface of interaction between the bone tissue and the sample with image (d); (c) new bone tissue forming within the sample surrounded by native bone cells; (d) the remains of the IBS was surrounded by native bone cells.

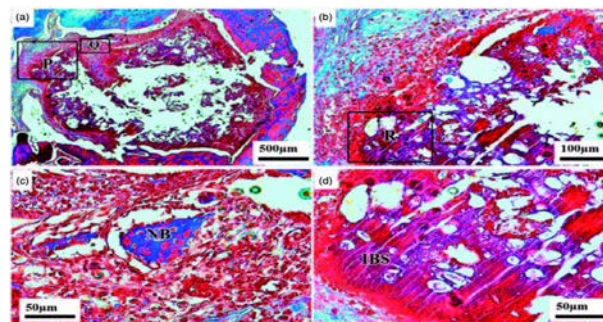


Fig. 1: Histological sections of implanted IBS-BG0 sample in a Rabbit's femur stained using Masson's trichrome staining method.

The setting time decreased and compressive strength with addition of Bioglass as shown here.

Table 1. Setting time & compressive strength of the Bioglass loaded IBS

	Setting time (min)	Compressive strength (MPa)
0% BG	42	19
10% BG	36	26
20% BG	27	34
30% BG	25	36

DISCUSSION & CONCLUSIONS: With the increase of bioactive glass content, the biocompatibility of the bone substitute sample increased. No inflammatory reaction was detected from the sampling site of the IBS. New bone formations were observed and that remains of the IBS were found to be surrounded with native bone cells indicating good interactions between the IBS samples and the native bone tissues.

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Effects of recombinant human bone morphogenetic protein-2 on bone regeneration of fibrous BCP-PCL/GEL composite

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INTRODUCTION: To confirm the effect of recombinant human bone morphogenetic protein-2 (BMP-2) for bone regeneration, BMP-2 loaded polycaprolactone (PCL)-gelatin (Gel)-biphasic calcium phosphate (BCP) fibrous scaffolds were fabricated using the electrospinning method. Using osteoinductive growth factors to enhance bone reconstruction and formation is the most efficient way to perform protein therapy [1,2]. We investigated the effects of BMP-2 on cell proliferation and differentiation of MC3T3-E1 cells and focused on osteogenesis in a rat calvarial skull model.

METHODS: To fabricate the electrospun scaffolds, 10% w/v PCL and 10% w/v Gel solutions were prepared by dissolving 1 g of each polymer in TEF separately. The 10% w/v Gel solution was added to the 10% w/v PCL solution at a volume 50:50 ratio of PCL:Gel. BCP particles were dispersed in TFE by ultrasound and added to the PCL-Gel solution at a ratio of BCP: PCL: Gel = 1:1:1 (w/w). In addition, 0.5µg/ml BMP-2 was mixed with the PCL-Gel-BCP solution before starting the electrospinning process. The PCL-Gel-BCP scaffolds and BMP2 loaded PCL-Gel-BCP scaffolds were fabricated by an electrospinning method. The scaffolds characterization was investigated by SEM and XRD. In-vitro and in-vivo experiments were performed to evaluate osteogenesis.

RESULTS: BMP-2 was homogeneously loaded on the PCL-Gel-BCP scaffolds for enhanced induction of bone growth. In the initial stage, BMP-2 was released at high levels, and then showed sustained release behavior for 31 days. Compared with the PCL-Gel-BCP scaffold, the BMP-2 loaded PCL-Gel-BCP scaffold showed improved cell proliferation as well as cell adhesion behavior. Both scaffold types were implanted in rat skull defects for 4 and 8 weeks to evaluate the biological response under physiological conditions. H&E staining of the tissue sections revealed significantly different amounts of new bone formation in the groups. The defect zone of the control group only had fibrous connective tissue at 4 weeks. In

contrast, the PCL-Gel-BCP and BMP-2/PCL-Gel-BCP groups revealed newly formed bone and dense connective tissue from the edge of the interface between the scaffolds and the host bone. At 8 weeks, remarkable bone regeneration was observed in the BMP-2/PCL-Gel-BCP group.

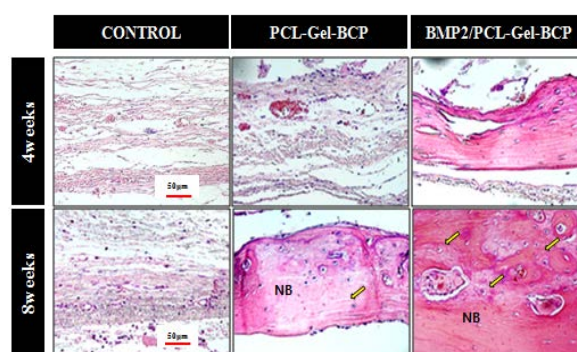


Fig. 1: Histological sections of implanted electrospun scaffolds (control, PCL-Gel-BCP-BMP2/PCL-Gel-BCP) in a rat's skull stained using hematoxylin & eosin method.

DISCUSSION & CONCLUSIONS: A novel BMP-2/PCL-Gel-BCP fibrous scaffold hybrid system combining ceramic/polymers and growth factors was fabricated using an electrospinning process. The in vitro study showed that BMP-2 was released from the scaffolds with appropriately sustained release behavior, which positively influenced cell behavior and differentiation. The release of BMP2 helped enhance bone formation at the early stage. Our results suggest that BMP-2/PCL-Gel-BCP electrospun scaffolds can be used for bone tissue regeneration and local delivery of biomolecules.

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Material characterization and in-vitro biocompatibility of HAp scaffold with in-vivo deposited extra cellular matrix (ECM) for bone tissue engineering

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INTRODUCTION: The aim of this study is to produce a sponge scaffold with inherent extracellular matrix (ECM) for bone tissue regeneration without employing the usual hydrogel coating or loading method. Material characterization such as compressive strength, porosity and surface morphology of bare, extracted and decellularized scaffolds were evaluated. In-vitro biocompatibility was also determined by observing cell proliferation and cell adhesion behaviour of MC3T3-E1 pre-osteoblast cell seeded in decellularized constructs.

METHODS: Polyurethane foams measuring 8 mm x 8 mm x 5 mm thick were dipped in HAp slurry and pressed gently to remove excess slurry. Air pressure was applied using an airgun to keep the pores open and to aid the penetration of slurry into inner portion of polyurethane foams. After coating, PU foams were dried in an oven at 80°C for one hour. Coating procedure was repeated 5 times and sintered at 1350°C for 16 minutes using microwave sintering oven. HAp scaffolds were implanted in the subcutaneous pockets of Sprague-Dawley rat for 1 week and 2 weeks. Animals were sacrificed after 1 week and two weeks implantation and further processed and analyzed. Extracted scaffold were washed using PBS thrice, soaked in SDS solution and incubated at 37°C for 48 hours with agitation. SDS solution was discarded every 12 hours to ensure complete removal of discarded cells. After 48 hours, scaffolds were soaked again in SDS solution and sonicated at 37°C for 10 minutes.

RESULTS: Cell adhesion and proliferation of MC3T3-E1 were evident in bare, 1 week decellularized and 2 week decellularized

scaffold with 2 week decellularized scaffold has the best cell adhesion and proliferation as seen in Fig. 1

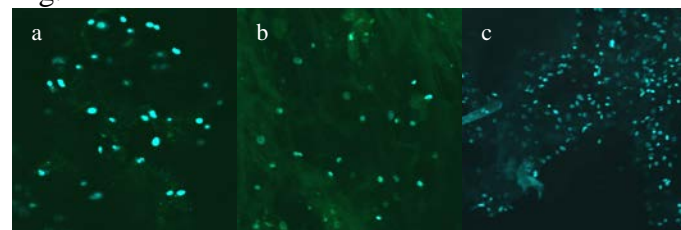


Fig. 1: Cell proliferation and adhesion of MC3T3-E1 on bare (a), 1 week decellularized (b) and 2 week decellularized scaffold.

Table 1. Compressive strength and porosity of bare, extracted and decellularized scaffold (1 & 2 weeks)

	Bare	Ext 1W	Ext 2W	Decell 1W	Decell 2W
Compressive Strength (MPa)	2.8	3.0	7.8	1.9	2.5
Porosity (%)	66.5	51.5	47.8	51.9	50.7

DISCUSSION & CONCLUSIONS: The longer time of in-vivo ECM deposition increases compressive strength while porosity decreases in both extracted and decellularized scaffolds. Decellularized in-vivo ECM deposited scaffold proved to be biocompatible based on cell adhesion and proliferation of MC3T3-E1 pre-osteoblast cells. Increased in cell proliferation and better adhesion were seen on 2 week decellularized scaffolds among three samples.

REFERENCES: ¹Badylak SF, Tullius R, Kokini K, Shelbourne KD, Klootwyk T, Voytik SL, et al. (1995) *J Biomed Mater Res* **29**:977–85.

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Hydroxyapatite-based artificial dental root for assessing root canal filling

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INTRODUCTION: Restoration and conservation of teeth constitute the bulk of treatment to patients in general practice. In conservative dentistry, endodontics (root canal treatment) deals with the dentin-pulp complex and requires the use of delicate instruments in confined spaces [1]. The goal of obturation is to provide an impermeable fluid tight seal within the entire root canal system. Broken or immature roots particularly require injectable calcium silicate cement filling. The success of root canal treatment depends on the mechanical, physical and biological properties of obturation materials. Development of new filling materials demands a great amount of dental roots to assist the research.

However, the use of human teeth is hindered by great variations with anatomy, age, and pathological history of the teeth, in addition to other drawbacks, including restricted access to extracted teeth due to ethical considerations, cross-infection risks, *etc.* Therefore, development of artificial root model with similar characteristics to natural teeth is highly desired.

In the present study, we developed a hydroxyapatite-based artificial dental root with similar composition and microstructure to dentin and similar internal anatomy to root canal system.

METHODS: *Fabrication of artificial dental root.* Hydroxyapatite (HA) was synthesized by aqueous precipitation method. Rice starch crystal, as porogen, was added to HA powder to obtain a microporous artificial dentin. Basing on the micro computed tomography (microCT) data, wax model of natural tooth root canal system were reproduced by computer aided design/computer aided manufacturing and plaster-casted into green body of root, which was then sintered at 1275°C to completely crystallize HA and eliminate porogen and wax.

Verification of internal morphology of artificial root. In order to compare internal anatomy, four reproduced artificial roots were scanned by microCT at 80 kV and 100 μ A with an isotropic resolution of 20 μ m. Images were registered by DataViewer[®] software, and

volumes of interest were determined for 500 slices from the apex with CTan[®] software. The series were imported in 3DSlicer software, and a fixed threshold was applied to select internal volumes for their reconstruction. Dice-Sørensen similarity coefficient and Hausdorff's distances were processed to compare morphology of different duplicated roots.

RESULTS: Sintering at 1275°C and addition of starch resulted in a similar microstructure in terms of porosity and pore size to natural dentin. By comparison of internal morphology of reproduced artificial roots (Fig. 1), the average Dice-Sørensen coefficient was 0.897 ± 0.025 , which shows a high similarity and reproducibility of fabrication process. Hausdorff's distances were found as 0.4 ± 0.086 mm, which indicates the presence of small defects such as minor deformations or voids.

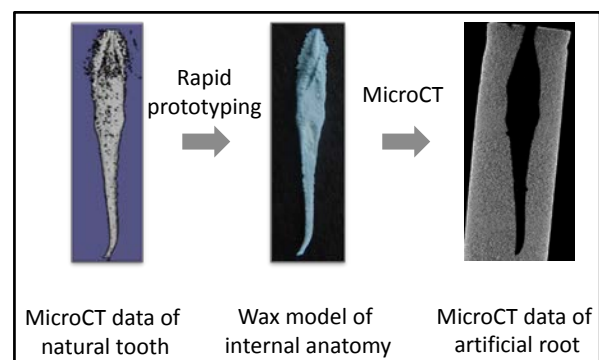


Figure 1: Reproduction and verification of internal morphology of tooth root canal system.

DISCUSSION & CONCLUSIONS: This developed artificial dental root has similar characteristics (composition, microstructure, internal anatomy, *etc.*) to natural tooth. The fabrication process was proved highly reproducible to prepare a suitable model in large quantities. *In vitro* assessment or comparison of endodontic injectable materials in this artificial root model will be conducted in next step.

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