SETTING TIME AND INITIAL COMPRESSIVE STRENGTH OF A RADIO-OPAQUE PREMIXED CALCIUM PHOSPHATE CEMENT

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INTRODUCTION

Calcium phosphate cements (CPC) have many uses in orthopaedics as bone void fillers. There are many products on the market intended for various indications[1]. CPCs have very good biological properties and stimulate bone ingrowth while being resorbed. However the handling of CPCs poses a problem. They must be mixed at the time of surgery and setting starts immediately after powder and liquid have been mixed, resulting in a limited working time. The handling is complicated and filter pressing often occurs. To solve this issue water can be exchanged for glycerol as mixing liquid[2]. This allows for a virtually unlimited working time since the setting does not start until the cement is inside the body where it comes into contact with water. There are very few CPCs intended for treatment of vertebral compression fractures. For this indication good injectability and radio-opacity are very important. $BaSO_4$ or ZrO_2 are often used as radio-opacifying agents in polymer based bone cements.

The aim of this work was to evaluate the influence of zirconia on the strength, setting time and radio-opacity of premixed calcium phosphate cement.

MATERIALS AND METHODS

The powder part of the cement consisted of β-tricalciumphosphate (β-TCP), monocalcium phosphate monohydrate (MCPM) and ZrO_2. MCPM and β-TCP were mixed in equimolar amounts. To this 0, 10, 20, 30, or 40 % (w/w) ZrO_2 was added. The powder was then mixed with glycerol. In all evaluated compositions the powder/liquid ratio was 4.2 g/ml. After mixing the paste was injected into 6x12 mm cylindrical moulds for compression strength testing or 6x3 mm cylindrical moulds for setting time. To initiate the setting of the cement the moulds were immersed in 37 °C PBS. Setting time was evaluated using the Gillmore needle method. For x-ray opacity measurements 1 mm thick samples were produced. The x-ray opacity was measured at 1 mAs, with 40 and 80 kV. A 1 mm sample of Vertebroplastic (PMMA based cement) was used as control along with an aluminium wedge.

RESULTS

An increasing amount of ZrO_2 in the cement pastes increases the liquid to cement ratio. This means that the reactive component of the cement needs to cement a larger volume for a high strength material to form. Accordingly the compressive strength was reduced from 13,3 to 7,9 MPa with an increase of ZrO_2 from 0 to 40% ZrO_2. The setting time was increased with an increasing amount of ZrO_2. 0% ZrO_2 gave a setting time of 20 minutes compared to 37 minutes with 40% ZrO_2. With 20 % ZrO_2 the compressive strength was 11,9 MPa and the setting time 25 minutes. From the X-ray images it could be concluded that 20 % ZrO_2 gave a sufficient radio-opacity, comparable to 1,5 mm aluminium at 40 kV.

CONCLUSION

The premixed calcium phosphate cement containing 20 % ZrO_2 was found to have excellent handling, good radio-opacity and sufficient strength and setting time.

ACKNOWLEDGEMENTS

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A NEW METHOD FOR CHARACTERIZING THE INJECTABILITY PROPERTIES OF HYDRAULIC BONE CEMENTS

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INTRODUCTION

It is important to find methods to characterize injectable bone cements to understand how they work during application. The objective of this study was to examine the potential of a specially designed Step-test as an addition or alternative to injectability tests. Two calcium sulphate based materials with different injectability properties were examined.

METHODS

The two materials (Cement 1 and Cement 2) used in this study were two calcium sulphate based injectable bone cements (60wt% calcium sulphate, 40wt% hydroxyapatite, added to an iodine containing liquid) with similar particle size and setting behavior but different injectability properties.

Initially the two cements were characterized using an injectability method, which simulates injection in thin duct systems, e.g. cancellous bone [1]. The cement paste was in this method extruded through thin cannulae. The extrusions were made between 3 and 12 minutes. At one-minute intervals the thinnest possible cannula to extrude from was evaluated. Then the two cements were characterized rheologically by using a specially designed Step-test which simulates the repeating extrusions during the injectability test (Anton Paar, MCR301 rheometer). The measurements were performed between 2 and 14 minutes increasing the strain stepwise from 0.05% to 50% strain at 5 different time points.

RESULTS

The injectability method showed that Cement 2 was more difficult to inject than Cement 1. Larger cannulae diameters (mean Dinner=0.44mm) were needed and injection stops occurred frequently during the test. Cement 1 was easier to extrude and only small cannula diameters were needed (mean Dinner=0.21mm). The Step-test (Fig.1) confirmed that there is a difference between Cement 1 and Cement 2. Especially during the deformation intervals, which represent the injection through cannulae, it could be seen that the two materials had different properties. Cement 1 was, in most cases a viscoelastic solid even under deformation while Cement 2 changed its inner structure directly from a viscoelastic solid to a viscoelastic liquid as soon as the deformation started.

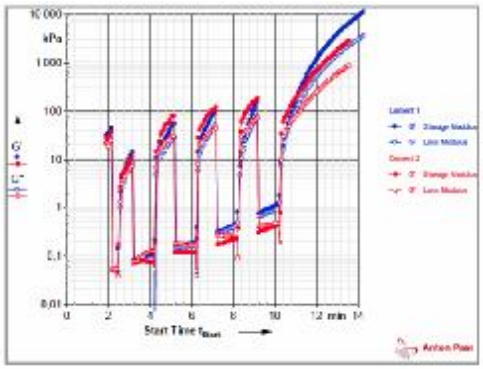

Fig.1*: Step-test results for Cement 1 and 2*

CONCLUSIONS

This study has shown that the designed rheometer Step-test shows the same results as the injectability method. Cement 1 and Cement 2 have different injectability

properties. The Step-test gives more objective and controlled information plus additional information about the differences between the two materials. In this case they cannot handle deformation in the same way. To summarize the Step-test is a promising alternative to injectability tests.

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INJECTABILITY OF ZN AND ZNSR-SUBSTITUTED b-TCP BRUSHITE FORMING CEMENTS

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INTRODUCTION

The continued increase in the age of the population and the concomitant increasing incidence of bone diseases such as osteoporosis, osteomyelitis, malignant tumors, or traumatic accidents, has generated higher demands for bone grafting.

Injectable CPC can fit bone defects perfectly and be used as an adjunct to internal fixation for treating selected fractures, as filling voids in metaphyseal bone, thereby reducing the need for bone graft, and improving the holding strength around metal devices in osteoporotic bone.

At present, there are two types of CPCs depending on the end-product of the reaction: apatite cements and dicalcium phosphate dihydrate (DCPD or brushite) cements. Brushite cements have raised special interest because they are resorbed *in vivo* much faster than apatite cements and brushite based cements possess faster setting reactions.

In recent years, ionic incorporations in b-TCP ceramics, such as zinc (Zn) and strontium (Sr) have been the subject of massive interest owing to the critical role of these ions in the biological process after implantation. Zn is an essential trace element for promoting osteoblastic cell proliferation and differentiation and thought to possess a potent and selective inhibitory effect on osteoclastic bone resorption *in vivo*. Sr has beneficial effects in the treatment of osteoporosis due to the prevention of bone loss by mechanism of depressing bone resorption and maintaining bone formation.

The present study focused on investigates the influence of Zn and ZnSr-substitutions on the rheology and injectability of brushite-forming cements.

MATERIALS AND METHODS

The CPCs were prepared from a mixture of powders consisting of 55 wt.% of Zn- or ZnSr-substituted b-tricalcium phosphate (b-TCP) + 45 wt.% of monocalcium phosphate monohydrate (MCPM) and an aqueous solution of 15% Citric Acid + 10 wt.% poly(ethylene glycol) (PEG 200) + 0.5 wt.% hydroxylmethylcellulose (HPMC). The substituted b-TCP precursor was obtained by an aqueous precipitation method from $Ca(NO_3).4H_2O$, $(NH_4)HPO_4$, $Zn(NO_3)_2$ and $Sr(NO_3)$.

The liquid-to-powder ratio was in the range of 0.34 – 0.36 mL g^{-1} at room temperature.

XRD and structural analysis by the Rietveld refinement method were employed to characterize the powders. The pastes were characterized by rheological measurements. Injectability was determined as the mass of the paste that could be expelled from a 10mL syringe divided by the original mass of the paste inside the syringe.

RESULTS

Quantitative Rietveld analysis of XRD patterns confirmed that the starting powders consisted of 100% of b-TCP

phase, while the cements contained around 90% of brushite and about 10% of b-TCP. The results showed the successful ionic incorporation into β-TCP structure by changes of the lattice parameters.
Dynamic rheological studies indicated that the CPC pastes presented the structure similar to viscoelastic body and the property of shear thinning at the beginning. The present study also showed that the obtained doped cement pastes exhibited general good injectability characteristics even under a moderate maximum applied force of 100 N.

CONCLUSIONS

The present work shown that the doped brushite-forming cements especially the ZnSr-substituted one, hold an interesting promise for uses in trauma surgery such as for filling bone defects and in minimally invasive techniques.

SELF-HARDENING HYDROXYAPATITE FOAMS FOR MINIMAL INVASIVE BONE GRAFTING

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INTRODUCTION

Calcium phosphate cements (CPCs) are suitable materials for bone grafting applications via minimal invasive surgical techniques. Recently different processes have been developed to obtain macroporous CPCs. Nevertheless, no all this methods result in injectable materials. The present work deals with the development of macroporous CPCs obtained by liquid phase foaming method, which are suitable for minimal invasive bone grafting applications. A synthetic surfactant, Polysorbate 80, whose commercial name is Tween 80, and an amphiphilic protein, gelatine, are compared as foaming agents of a CPC.

EXPERIMENTAL METHODS

The powder phase of the CPC used in this study consisted of 98 wt% alpha tricalcium phosphate (α-TCP) and 2 wt% of precipitated hydroxyapatite (HA). The two foaming agents studied were Polysorbate 80 (Sigma-Aldrich, UK) and bovine gelatine type B (250 Bloom; Rousselot, France). Each of them was incorporated to the cement liquid phase at concentrations of 1 wt%. The liquid phase was a 2.5 wt% Na_2HPO_4 aqueous solution (Merck, Germany). The disodium hydrogen phosphate was used as an accelerant of the setting reaction, to guarantee adequate setting times. Self-setting calcium deficient HA (CDHA) foams were obtained by foaming 2 ml of the liquid phase during one minute and careful mixing with the solid phase at different liquid to powder (L/P) ratios (0.32, 0.45, 0.55, 0.65 and 0.80 ml/g), where the volume corresponds to the liquid phase prior to foaming [1,2]. The cohesion of the foamed pastes was determined by visual inspection of the paste immersed in water at 37 °C, and the injectability was measured as the relative amount of paste that could be extruded trough a syringe with a 2 mm aperture up to a maximum load of 100 N. The test was performed at room temperature in a universal testing machine (MTS Bionix 858, USA), at a cross-head speed of 15 mm/min. The porosity and pore interconnectivity of the CDHA foams was measured by mercury intrusion porosimetry (MIP, Micromeritics AutoPore IV 9500, USA). The microstructure was also analysed by scanning electron microscopy (SEM, Jeol JSM 6400). The conversion of the

α-TCP into CDHA was assessed by X-ray diffraction (Simens D-500).

RESULTS

All foamed cement pastes were able to set in simulated body conditions, resulting in solid CDHA foams. The total porosity and the porosity induced by the foaming process increased with the L/P ratio for both surfactants, and it was higher for Polysorbate 80 than for gelatine, the difference among the two surfactants increase also with the L/P ratio. The pore size distribution of two samples with similar total porosity as measured by MIP is shown in Figure 1a, where the CDHA foam obtained with 1 wt % gelatine at a L/P ratio of 0.80 ml/g and that prepared with 1 wt % Polysorbate 80 at a L/P ratio of 0.55 ml/g are compared. SEM images of the two solid CDHA foams are compared in Figure 1b) and c). Spherical macropores were observed in both cases, with larger diameters in the Polysorbate 80 foam than in the gelatine foam. Especially in the Polysorbate based foam numerous windows were observed between macropores, in agreement with the higher open macroporosity in the range of 10-360 μm assessed by MIP. The injectability of the foams depended also on the L/P ratio and the foaming agent incorporated. Even if the injectability of both types of foams was high, the injectability of the gelatine containing foam was higher than that of the Polysorbate 80 one. The foams were completely injectable when were prepared at L/P ratios of 0.50 ml/g for Polysorbate 80 and 0.75 ml/g for gelatine. Moreover, the foams were able to retain the porous structure after injection provided that the foamed paste was injected shortly after mixing.

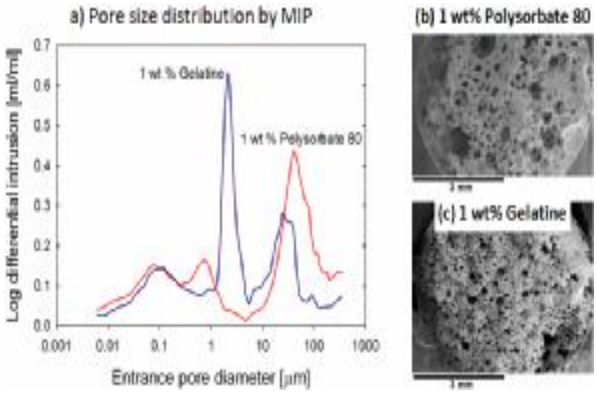

***Fig.1**: a) Pore size distribution obtained by MIP for the Polysorbate 80 and gelatine CDHA foams; b,c) respectively Polysorbate 80 and gelatine CDHA foam macrostructure. In all cases the quantity of the foaming agent is 1 wt%.*

CONCLUSION

Foaming the liquid phase of a CPC allows obtaining injectable self-hardening CDHA foams for bone grafting applications. The selection of the foaming agent is a critical factor in this process. Polysorbate 80 is a more efficient foaming agent than gelatine. However, gelatine improves the foamed paste injectability and cohesion, both essential properties in minimal invasive surgery.

ACKNOWLEDGMENTS

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IN-VITRO STUDY OF CALCIUM PHOSPHATE-CALCIUM CARBONATE INJECTABLE CEMENT LOADED WITH STRONTIUM AS SUSTAINED RELEASE SYSTEM

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INTRODUCTION

Injectable bone cements are good candidates for mini-invasive surgery techniques but need to be radio-opaque to enable surgeons to easily follow their introduction into bone defects. As calcium phosphate cements (CPC) are not intrinsically enough opaque, there is a need for a non-toxic contrast agent, especially in materials that generate resorption activity. In this view, strontium has recently been proposed to be added in CPC formulations [1]. Moreover, other studies have shown its beneficial effect as a therapeutic agent on bone reparation [2]. In fact, at low doses it has a synergistic effect on osteoblasts and on osteoclasts. However it seems that strontium has deleterious effects on the bone when present at high doses. Therefore, this study aims to introduce strontium in a calcium carbonate-based cement formulation which has been recently presented as a promising biomaterial for bone reconstruction [3] and investigate *in-vitro* its strontium release properties. The daily released rate of strontium will particularly be studied to ensure both its beneficial effect on bone reparation and a sustained release.

MATERIALS AND METHODS

Cements have been prepared by mixing deionised water as a liquid phase (L) with the solid phase (S) constituted by a mixture of $CaCO_3$-vaterite and dicalcium phosphate dihydrate. Strontium was introduced in the cement paste either as $SrCO_3$ in S, or as $SrCl_2,6H_2O$ dissolved in L. In every case, L/S was equal to 0.5 (w/w) and the paste was introduced into a mould to set and harden at 37°C. Then, cement blocks were dipped in a stirred tris(hydroxymethyl)aminomethane solution (0.1 M) at pH=7.4 and T=37°C using a USP rotating paddle apparatus (Dissolutest, Pharmatest). Samples of the solution were withdrawn periodically during 21 days and concentrations of Ca^{2+} and Sr^{2+} were determined using the atomic absorption spectroscopy. Cements were characterized by FTIR spectroscopy, X-ray diffraction and SEM techniques before and after release test.

RESULTS

Depending on the way strontium was introduced into the cement paste (in S or in L), set cements have different compositions and therefore different behaviours in solution. When $SrCO_3$ is introduced into S, it does not take part in the setting reaction and the major phenomenon responsible for Sr release is diffusion, as confirmed by Higuchi model. However, when $SrCl_2$ is introduced into L, Sr ions take part in the setting reaction and are incorporated within the apatite lattice limiting their diffusion through the cement. In this case, Ca^{2+}/Sr^{2+} ratio in the release-test solution was equal to the initial Ca/Sr ratio in the cement indicating a Sr release mainly due to an erosion process. We showed that there was an initial burst (during the first 3 days), after which the rate was slow and sustained. From days 1 to 3, the relatively high amount of strontium released should stimulate the osteoblasts to promote early bone formation; then, the sustained release will help the long-term reconstruction.

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THE STUDY OF CROSS-TALKING BETWEEN ERK AND SMAD PATHWAY IN CHONDROGENESIS OF MSCS INDUCED BY TGF-b3

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OBJECTIVE
To investigate the role of cross-talking of Erk and Smad signal pathway in the induction of marrow stromal cell (MSC) differentiation into chondrocytes by TGF-β3 and the signal network that is involved in chondrogenesis.

METHODS
Rat MSCs were induced to chondrocytes in DMEM supplemented with TGF-β3. At different time points (0, 0.5, 1, 2, 4, 8, 16, 32, 64 hours), the unphosphorylation and phosphorylation of ERK1/2 were detected with Western blot and the expression of genes related to chondrogenesis was analyzed. We also observed the changes of the chondrogenesis induced by TGF-β3 after using ERK1/2 inhibitor, UO126 to block the ERK1/2 signaling. Furthermore, we performed the mutation on the Smad4 gene to transfect MSCs and studied its effect on the chondrogenesis induced by TGF-β3.

RESULTS
Our study revealed that ERK1/2 was involved in the chondrogenesis and synthesis of cartilage matrix induced by TGF-β3. Use of UO126 can reduce the progress of chondrogenesis significantly, and the mutation of Smad4 at the phosphorylation sites (Thr277) of ERK1/2 showed similar effects of UO126. The above results revealed that ERK1/2 signal transduction pathway plays an important role in the chondrogenesis and this function may be associated with the phosphorylation of Smad4.

CONCLUSION
The phosphorylated Smad4 may transfer R-Smad to nucleus more efficiently, and the cross-talking between ERK1/2 and Smad signal pathway can enhance the regulation of signal transduction pathways mediated by TGF-β3.

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OSTEOGENIC PROPERTIES OF ZN AND ZNSR-SUBSTITUTED BRUSHITE CEMENTS

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INTRODUCTION
Calcium phosphate cements (CPC) have unique characteristics for bone substitution compared with other biomaterials. Their excellence relies on good biocompatibility, excellent bioactivity, self-setting characteristic, low setting temperature,

adequate stiffness, and easy shaping for any complicated geometry.
Brushite based bone cements are generally well tolerated by the bone and soft tissue environment *in vivo*, such that cement resorption is closely followed by new bone formation. Furthermore, brushite cements are known to be biocompatible, osteoconductive and bioresorbable, having a potential interest for bone regeneration procedures.
The ionic composition of biomaterials is a key factor in their bioactivity, and ionic incorporation into TCP structure, such as zinc (Zn) and strontium (Sr) ions, have been proved to be traces elements with stimulatory outcomes on bone formation, having a direct specific proliferative effect on osteoblastic.
The aim of the present study was to examine the *in vivo* response of Zn and ZnSr-substituted brushite cements, after implantation in trabecular bone in pigs. Additionally, the *in vitro* proliferation and differentiation responses of MC3T3-E1 cell line to the brushite cements were assayed, including cell viability determinations by the resazurin assay, photometric evaluation of ALP activity.

MATERIALS AND METHODS

Brushite cement pastes were produced by mixing 55 wt.% of Zn- or ZnSr-substituted b-tricalcium phosphate (b-TCP) powder with 45 wt.% of monocalcium phosphate monohydrate (MCPM) using liquid-to-powder ratio of 0.34 mL g^{-1}. The mixing liquid used was 15 wt.% citric acid solution + 10 wt.% poly(ethylene glycol) (PEG 200). Powders were obtained by an aqueous precipitation method from $Ca(NO_3).4H_2O$, $(NH_4)HPO_4$, $Zn(NO_3)_2$ and $Sr(NO_3)$.
The setting times of the cements were assessed using Gilmore needles. Mechanical strengths of the cements were also evaluated.
The osteoblastic MC3T3-E1 cell line was used for *in vitro* studies and the resazurin metabolic assay was used to determine the cements cytotoxicity/biocompatibility.
Histological and histomorphometric analyses were performed using a laser scanning confocal fluorescence microscope.

RESULTS

Initial and final setting times of the cement pastes showed that ZnSrCPC sets faster than ZnCPC. The measured values of the wet strength after 48 h of immersion in PBS solution at 37ºC showed that ZnSrCPC cements are stronger than ZnCPC cements.
Presence of Zn and Sr in the studied cements was found to stimulate pre-osteoblastic proliferation, activity and maturation. MC3T3-E1 osteoblastic-like cells exposed to the powdered cements showed higher adhesiveness capacities and higher ALP activity, in comparison with control cells.
The studied cements injected into trabecular bone defects in pigs proved that Zn and Sr are good inductors of osteoprogenitor cell proliferation and differentiation, without evidence of adverse foreign body reaction. Histological and histomorphometrical analyses revealed regeneration of new bone and a gradual penetration into the implant. Also, results indicated that Sr is a more potent inhibitor of osteoclastic activity than Zn, as much fewer osteoclast-like cells could be found in ZnSr-containing implants.

CONCLUSIONS

The present work demonstrates that the investigated Zn and ZnSr-containing brushite cements are biocompatible, osteoconductive, and good candidate materials to use as bone substitutes.

INJECTABLE BIODEGRADABLE COMPOSITES FOR THE DEGENERATED INTERVERTEBRAL DISC

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INTRODUCTION

Treating the degenerated intervertebral disc is a challenge for surgeons and biomedical engineers looking for methods to reduce the low back pain. Current solutions such as interbody fusion, partial and total disc replacements may give rise to several short and long-term problems regarding extrusion of the implants and further bone resorption [1,2]. This has prompted researchers to look for alternatives such as tissue-engineered intervertebral discs [3]. The requisites for an intervertebral disc substitute material are adequate mechanical properties, injectability, *in situ* crosslinking, and biodegradability if intended as a regenerative scaffold. The aim of this work was to perform a preliminary study on the usefulness of this type of material.

MATERIALS AND METHODS

Injectable crosslinkable oligomers of poly(D,L-lactide-co-caprolactone) were prepared via ring-opening polymerization of D,L-lactide and ε-caprolactone followed by end-capping with acryloyl chloride. The oligomer paste product was then mixed with particles of β-tricalcium phosphate and calcium carbonate and cured at room temperature via radical initiation to form solid specimens. The structure of the oligomers was determined by ^{1}H NMR spectroscopy. Furthermore, mechanical testing, BET analysis, XRD and electron microscopy were performed to characterize the materials. Finally, an *in vitro* degradation test was carried out to study the degradation mechanisms of the final composites.

RESULTS

The oligomers were successfully synthesized and both fillers were well dispersed in the matrix after manual mixing. The presence of ceramic particles increased both the compressive modulus and the maximum compressive strength. Furthermore, $CaCO_3$ and β-TCP particles reduced the degradation rates of the composites when compared to the pure polymer. This was accredited to carboxylic end-group stabilization and the buffering-effect due to the Ca^{2+} ions released by the ceramic dissolution. Due to polymer interpenetration, porous β-TCP showed a better mechanical attachment to the polymer matrix than $CaCO_3$. However, the composites containing $CaCO_3$ (calcite) exhibited bioactive properties by growing an apatite-like layer on its surface when immersed in PBS solution. The presence of Ca and P was confirmed by EDX analysis.

CONCLUSION

In situ crosslinkable biodegradable composites were successfully prepared. These composites show potential as augmentation materials or scaffolds for intervertebral disc regeneration.

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NEW BISPHOSPHONATE-LOADED CALCIUM PHOSPHATE CEMENT FOR THE LOCAL TREATMENT OF OSTEOPOROSIS: DESIGN AND CHARACTERIZATION

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INTRODUCTION

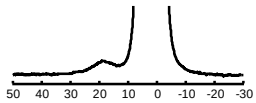
Association between Bisphophonates (BP) and Calcium phosphate Cement (CPC) to form a new drug delivery bone substitute for the local treatment of Osteoporosis through minimally invasive surgery, is a promising challenge. However, the way to introduce BP in a CPC has a critical influence on the setting kinetics. We have found suitable conditions to design the first example of BP-loaded CPC showing appropriate properties including injectability, setting time and drug release profile.

MATERIALS AND METHODS

The formulation was optimized from an apatitic-type CPC[1], with the following composition for the solid phase: a-TCP, dicalcium phosphate dihydrate, monocalcium phosphate monohydrate, BP loaded-calcium deficient apatite [BP-CDA], polysaccharide. The liquid phase consisted in a Na_2HPO_4 solution. The BP used for our study was Alendronate. BP-CDA was obtained by suspending CDA (1g) in 10 mL of a solution of Alendronate ($C= 0.5\ 10^{-3}$ - $2.5\ 10^{-2}$ $mol.L^{-1}$). The cement obtained was characterized by ^{31}P solid state NMR and high frequency impedance which are original methods for monitoring the CPC setting.

RESULTS

Three approaches to incorporating Alendronate in the cement were investigated : BP (i)dissolved in the liquid phase (ii) added to the ground solid phase (iii) chemically combined with a component of the solid phase. As phosphonates are considered as retardant agents for the setting of Portland cements, the later approach has been shown to be the most promising particularly via the chemical association between CDA and BP as previously demonstrated[2,3]. We have shown that the same association process was taking place in the case of our apatitic cement and BP. In this study we have also compared the setting time mesurements using Gillmore needles standard and high frequency impedance measurements which have proven to be effective for the the studies of solid/liquid interfaces. Comparison between the final setting t_f from Gillmore method with that evaluated from dielectric data suggested that the former was unadequate to characterize the setting reaction. The presence of Alendronate in the BP-loaded cement was demonstrated by NMR experiments which have been also used to characterize the chemical transformation of a-TCP to CDA during the setting process.



Left : Compararison between the Gillmore method and dielectric measurements.
Upper : ^{31}P NMR spectrum of BP-loaded cement showing the BP signal at 18.5 ppm

BP absorption/desorption experiments have been realized on cement blocks, under continuous flow condition, to model the release profile of the Alendronate in a medium close to the *in vivo* situation. No flash release was observed and the BP concentration was lower that the cytotoxic level (6.10^{-9}M).

CONCLUSIONS

This new BP-loaded CPC possessed the main suitable properties of an unloaded CPC (injectability, setting time, strength) with in addition, the capacity to release an osteoporotic drug in a controlled manner.

Pre-clinical studies using this combined system are currently underway.

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AUGMENTATION OF BURST FRACTURES
PART 1: DESIGNING AN OPTIMUM CALCIUM PHOSPHATE CEMENT

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INTRODUCTION

Burst fractures are traumatic vertebral fractures which are commonly treated using highly invasive spinal fusion. A minimally invasive technique known as vertebroplasty could often benefits over traditional options. This procedure involves injecting bone cement into the fracture site where the cement subsequently hardens allowing for fracture stabilisation. The current material of choice is PMMA, however due to it bioinert nature this is not the optimum material for younger patients with the potential of bone remodelling. Calcium phosphate cements (CPC's) could provide an alternative due to their resorption capabilities. However, they lack the mechanical integrity needed for this specific application. Therefore this study considers the use of an optimised CPC for the treatment of burst fractures using a porcine model.

EXPERIMENTAL METHODS:

An optimum cement composition was hypothesised and tested using a Design of Experiments (DoE) [1]. The design considered the effects of 5 factors to include the weight percentage (wt%) of Na_2HPO_4; liquid to powder ratio (LPR); type of HA; amount of HA and time in Ringer's solution. The cement was characterised using the following techniques. Compressive properties were tested in accordance with ISO 5833:2002. Injectability was measured by extruded the cement through a syringe at a constant extrusion rate of 10mm/min with a maximum load of 100N. Setting times were analysed using the Gillmore needle apparatus complying with ASTM C266-89. Burst fractures were then created in a three segment porcine model (n = 6) using a drop weight method [2]. Fractures were repaired using the vertebroplasty procedure with the optimum CPC. To establish cement distribution and injection capability the augmented porcine vertebrae were scanned in a microCT system (Scanco Medical, Switzerland).

RESULTS & DISCUSSION:

The DoE indicated the overall contribution of each factor on the measured properties, any factor with a contribution ≥10% was deemed significant. Compressive strength was greatly affected by the LPR, which contributed to 82%. Injectability was affected by the LPR (65%) and wt% of Na_2HPO_4 (14%). Setting times were affected by the LPR (32%) and wt% of Na_2HPO_4 (50%). An optimum cement composition was proposed which included; 100% α-TCP, 5wt% NA_2HPO_4, LPR of 0.35mL/g and stored for 5 days in Ringer's solution. The optimum CPC was then tested before injection into the porcine vertebrae (Table 1). The predicted values proposed by the DoE and the experimentally measured values differed. This variation is expected as the properties of the CPC system hypothesised by DoE did not take into account all factors, which are interrelated. This optimum cement

demonstrated a compressive strength within the range stated for vertebroplasty i.e. ≥ 30MPa [3].

Table 1: *Material Properties of Optimum CPC*

Property	***Predicted Value***	***Experimental Value***
Compressive Strength (MPa)	26	30.3 ± 4.7
Injectability (%)	30	52.6 ± 7.3
Final Setting Time (mins)	13	23 ± 0.9

During the vertebroplasty procedure, it was observed that the cement was easily injected into the vertebral column, with an injectability of 76.3 ± 7.1%, indicating good penetration into the fracture site. MicroCT analysis highlighted that the cement performed best in the more severe fractures (Figure 1). Moreover, microCT analysis indicated that a bipedicular method could have achieved better fracture stabilisation.

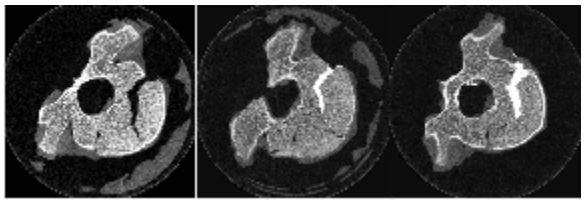

Figure 1: *Example of an Augmented Burst Fracture*

CONCLUSIONS:

This study was carried out to determine the performance of an optimum CPC within a porcine vertebra model. It highlighted that the optimal CPC could be injected into severe burst fractures. Future tests will consider cements with a range of viscosities to further investigate injection capabilities.

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AUGMENTATION OF BURST FRACTURES PART 2: MECHANICAL PROPERTIES OF AUGMENTED SPECIMENS

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INTRODUCTION

Burst fractures of the vertebral body are reported to account for about 15% of all spinal fractures [1]. Due to the nature of the injury it is prevalent in the younger population where there is the potential for bone healing. In recent years vertebroplasty using bio-inert polymer based bone cement has been highlighted as a possible minimally invasive treatment for the injury. However, calcium phosphate cements (CPC) exhibit more desirable biological compatibility and resorption characteristics. Due to this they have been proposed as a possible alternative to PMMA as an augmentation cement. This study examines the effectiveness of using a newly developed CPC as an augmentation cement to repair burst fractures in porcine vertebrae.

MATERIALS AND METHODS

Six three-vertebra porcine specimens were fractured using a drop weight method. The middle vertebra was extracted from each segment and stripped of excess tissue. Each endplate was encased in a removable PMMA housing to provide parallel faces for mechanical testing. A marker was fixed to the superior PMMA housing at the centre of the vertebral body, to identify the intended point for axial loading. The

specimens were then scanned in a microCT system (Scanco Medical, Switzerland) indicating the most appropriate angle and depth for needle insertion. A uni-pedicular vertebroplasty procedure was then carried out. A newly developed calcium phosphate cement, developed at Queens University Belfast using a design of experiments process to optimize material properties [2] was used for all experiments. The cement was prepared and collected in a MiniMix precision delivery syringe (Summit Medical). Cement was then injected using a screw mechanism allowing accurate control over the rate of delivery, this mechanism was used to minimise the effect of particle deposition and filtration occurring, and allow more cement to disperse into the fracture. The specimens were then scanned in the microCT system to assess the cement distribution in the vertebral body. Following scanning, the specimens were tested in compression to a load of 6000N. For comparison, a similar sized group of intact single vertebra specimens were also tested using the same cement mounting technique.

RESULTS AND DISCUSSION

It was found that the cement could be injected successfully into the specimens with more severe fractures, dispersing evenly into the fracture gap, figure 1, but for less severe fractures and for instances where the needle placement was suboptimal, the cement interdigitation into the trabecular structure was poor. An estimate of the vertebral body modulus was calculated using the specimen stiffness and dimensions. These moduli were compared to that of intact specimens' of an equivalent vertebral level using a t-test. The means and standard deviations of the two groups are shown in figure 2.

Following the mechanical testing the augmented specimens all exhibited signs of re-failure, whereas only one of the intact specimens showed any signs of failure when tested to 6000N.

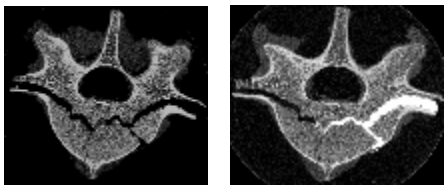

Fig.1: Section through Spine 2 L5 Vertebral body pre and post Augmentation (left, right)

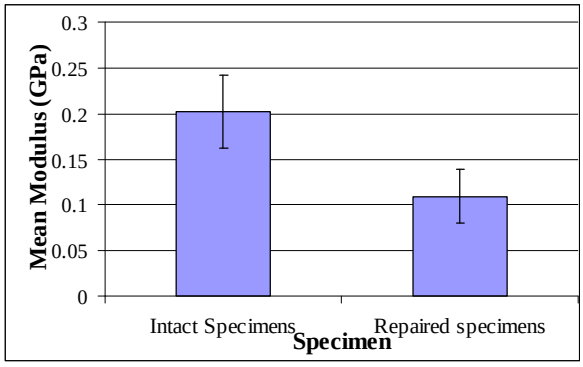

Fig. 2: The means and standard deviations of the repaired and intact specimen sets

CONCLUSION

The cement proved to be sufficiently injectable into specimens with large fracture gaps allowing cement to flow within vertebra. The results of the show that the specimens that have been fractured and repaired using the CaP cement have a significantly lower modulus when compared to the intact specimens. This suggests that the cement has not fully restored the stiffness of the intact vertebrae. The cement used in this experiment was optimized to yield the highest compressive modulus, further experiments will use a CPC cement with a lower viscosity to improve the chance of interdigitation into the trabecular structure. Further tests will compare the performance of two cements, and examine the difference between the treated and untreated cases. To compare with other cements clinically used in the medical field, a currently used PMMA cement will also be used for comparison. Finite element models of augmented and fractured vertebrae are now in development which will be used to develop a method of predicting vertebral stiffness

and failure strength following augmentation with CPCs.

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DETAILED EXPERIMENTAL CHARACTERISATION OF THE MATERIAL BEHAVIOUR OF BONE CEMENT FOR A THERMOMECHANICAL-CHEMICALLY COUPLED MATERIAL MODEL

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INTRODUCTION

Success in bone augmentation procedures depends much upon the experience of the physician and upon the cement behaviours. Heat necrosis, leaking monomer and contact of host tissue with the cement are side effects with potential complications. Numerical simulation using adequate constitutive model of the cement, prior to the cementation procedure, can provide the physician with guidance to effectively perform the intervention. Therefore, a precise characterisation driven by an accurate constitutive model that takes into account all the important effects is necessary.

EXPERIMENTAL METHODS

Time- and temperature-dependent exothermal curing behaviour of a commercial acrylic bone cement was studied using differential scanning calorimetry (DSC). The chemical shrinkage and thermal expansion were determined with a volumetric dilatometer. In addition, rheological experiments were used to provide information on the mechanical behaviour directly after cement paste.

The developed material model is able to describe the overall stress distribution by distinguishing thermal, chemical and mechanical deformations. Furthermore, the degree of cure and the temperature profile can be calculated for the entire curing process.

RESULTS

The cement curing behaviour has been described by means of reaction kinetics considering uncompleted curing. Chemical shrinkage due to polymerisation is approximately 7±SD % in volume but does depend on the incubation temperature, Fig. 1. The rheological properties showed a strong dependency on temperature and polymerisation. Right after cement mixing, the mechanical behaviour of the cement paste is dominated by a dissolution/swelling process of the PMMA particles [1].

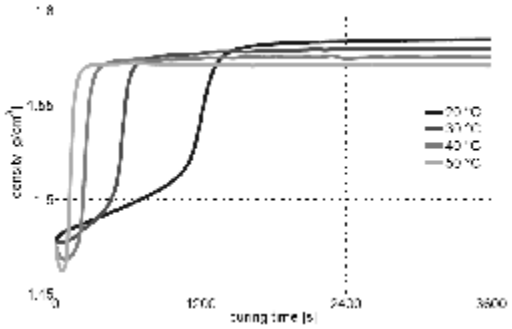

Fig 1: Shrinkage measurement

CONCLUSIONS

Having gathered the exothermal curing behaviour, mechanical properties, shrinkage and thermal expansion, a further step is to determine the thermal conductivity and the heat capacity of bone cement. These data will be required to

completely parameterise the constitutive model for use as a simulation tool that can be useful for physicians in performing the augmentation procedures.

ACKNOWLEDGMENTS
This research work is supported by the German Research Foundation (DFG) within the project "Numerical simulation of complex processes in minimal invasive stabilisation techniques of osteoporotic vertebrae"

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GALLIUM: A PROMISING CANDIDATE FOR A NEW BONE BIOACTIVE DRUG DELIVERY SYSTEM

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INTRODUCTION
Given that osteoporotic fractures mainly occur in the proximal femur, vertebral bodies and distal radius, their local prevention has recently been considered with interest. This strategy consists in locally reinforcing these specific bone sites by an implantable bioactive drug-combined biomaterial associating a calcium phosphate bone substitute with an inhibitor of osteoclastic activity. Among the potential compounds, gallium (Ga) could be a promising candidate due to its ability to substitute Ca in biological apatite. The chemical characteristics of Ga should presage the possibility of incorporating Ga into CaP biomaterials following an exchange between Ca and Ga. Interestingly, Ga is clinically used for the treatment of hypercalcemia in the case of malignancy and Paget's disease, thereby suggesting a potent inhibitory effect of Ga on bone resorption. However, the anti-osteoclastic effect of Ga still needs clear deciphering, given that the direct effect of Ga on bone cells has only been partially addressed.

EXPERIMENTAL METHODS
By using different osteoclastic models, osteoclasts isolated from long bones of neonatal rabbits (RBC), murine RAW 264.7 cells and human CD14-positive cells, we have performed resorption activity tests, TRAP staining, RT-PCR analysis, viability and apoptotic assays. Moreover, we have evaluated the effect of Ga on osteoblasts in terms of proliferation, viability and activity by using an osteoblastic cell line (MC3T3-E1) and primary osteoblasts.

RESULTS
Ga was found to dose-dependently inhibit the *in vitro* resorption activity of RBC. Ga also induced a significant decrease in the expression level of transcripts coding for osteoclastic markers in RAW 264.7 cells such as Tartrate resistant acid phosphatase (TRAP), Cathepsin K (CTK), Calcitonin receptor (CTR), Receptor activator of nuclear factor kappa B (RANK) and Osteoclastic stimulatory transmembrane protein (OC-STAMP). Ga also dramatically reduced the formation of TRAP+ multinucleated cells. Interestingly, Ga down-regulated in a dose-dependent manner the expression of NFATc1. Finally, our results indicate that Ga failed to dramatically affect the primary and MC3T3-E1 osteoblasts.

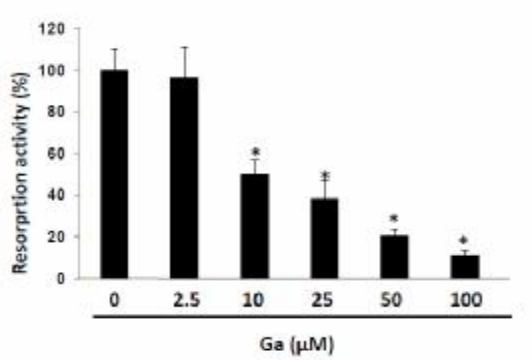

***Fig.1**. Effect of Ga on resorption activity*

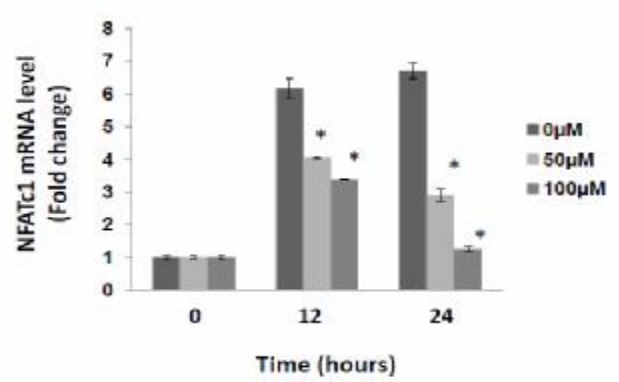

***Fig.2**. Effect of Ga on the mRNA expression of NFATc1*

DISCUSSION

This study demonstrates that Ga exhibits a dose-dependent anti-osteoclastic effect by reducing *in vitro* osteoclastic resorption, differentiation and formation without negatively affecting osteoblasts. This is the first report indicating that the inhibitory effects of Ga on osteoclastogenesis probably involve a reduction in the expression of NFATc1, a master regulator of RANK-induced osteoclastic differentiation. Considering these data, Ga appears to be a promising candidate to develop a new bone bioactive drug delivery system for the local reinforcement of osteoporotic sites.

IN-SITU INVESTIGATION OF TEMPERATURE INFLUENCE ON a-TCP CEMENT HYDRATION

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INTRODUCTION

Tricalcium phosphate Ca3(PO4)2 (TCP), an osteoconductive as well as bioresorbable phase, has found application as bone cement and bone implant material. TCP can crystallize in different polymorphic modifications of which the β-TCP (for T < 1180 °C) and α-TCP (for T between 1180 °C and 1430 °C) 1 are the most interesting for bone repair and bone regeneration respectively.

The hydration of α-TCP in aqueous solution to hydroxyapatite (HAP) was first reported in 1976 by Monma & Kanazawa2. In our study the hydration behaviour of α-TCP was investigated for short and longer milled powders with specific surface areas of 1.45 m²/g (α-TCP1) and 2.38 m²/g (α-TCP 2) with different amorphous content. The conversion of α-TCP into HAP was studied at 23 °C, 30 °C and 37 °C with heat flow calorimetry and in-situ-XRD. The results from both methods clearly show that at begin of the reaction hydration is controlled by the amount of XRD-amorphous α-TCP in the cements.

EXPERIMENTAL METHODS

α-TCP was synthesized by calcination of calcium carbonate and diammonium hydrogen phosphate. The purity of the α-TCP was controlled and confirmed by quantitative X-ray powder diffraction (XRPD) in combination with Rietveld refinements. In all powders the only crystalline phase was proven to be α-TCP. Rietveld refinement was performed using the structural models (ICSD Database) of all possible occurring secondary phases listed in Table 1. Refined parameters were scale factor, sample displacement, background as Chebyshev polynomial of 5th grade, crystallite size, micro strain and lattice parameters.

Table1: ICSD data for Rietveld refinements

Phase	Mineral/name	ICSD-Code
β-Ca2P2O7	β-C2P	14313
Ca10(PO4)6(OH)2	HAP (Hydroxyapatite)	99358
β-Ca3P2O8	β-TCP (Whitlockite)	97500
α- Ca3P2O8	α-TCP	923

Isothermal calorimetry investigations were performed with a TAM air heat flow calorimeter in an "AdMix-Ampoule" with a 0.2 molar Na2HPO4-solution with liquid to powder ratio of 0.65. The water was injected into the crucible and the originating slurry was stirred internally for 30 s.

In-situ X-ray diffraction investigations were carried out with a Siemens D5000 diffractometer with SolX detector. After preparation the samples were sealed with a Kapton® polyimide film to prevent evaporation of H2O and the potential reaction with atmospheric CO2.

RESULTS

The accelerating effect of longer milling times can clearly be shown by heat flow calorimetry. The increased reactivity is on the one hand caused by higher specific surface areas and on the other hand by the amount of defective or amorphous phase fraction. Combination of XRD- and calorimetric measurements (Figure 1) show that the amorphous α-TCP phase fraction is the first to be dissolved and that the second maximum in the heat flow can be directly correlated to the dissolution of crystalline α-TCP.

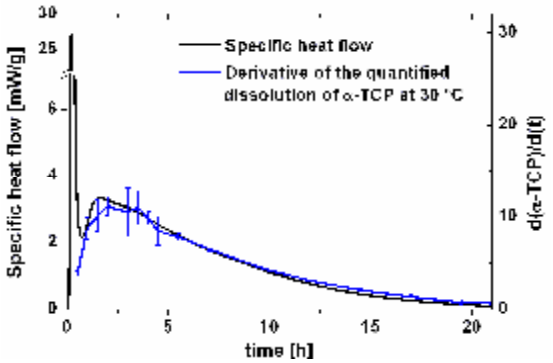

Figure 1: Derivative of the dissolved α-TCP with respect to the time and the correlated specific heat flow during the first 21 h of the hydration of α-TCP2 at 30 °C

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RESORPTION SIMULATION OF POROUS SUBSTITUTES FOR BONE REPAIR

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INTRODUCTION

Calcium phosphate bone substitutes are resorbable porous matrix to repair large bone defects. Simulating the resorption process is helpful in understanding the in-vivo behaviour of the resorbing substitutes. Recently we presented an algorithm to simulate the resorption process. The algorithm used μ-computed tomography (μCT) images and fuzzy image processing tools [1]. In continuity, this study aims at presenting the theoretical results of the resorption process to determine the resorption behavior and rate of the substitutes. Furthermore, in order to study the resorption from the periphery to the centre of bone substitute, the resorption rate was calculated at different regions of scaffolds.

METHODS

Four types of β-TCP substitutes with variable pore features and constant porosity (~50%) were scanned by a μCT system (Table 1). A scaffold structure was reconstructed using skeleton points and 3D fuzzy distance transform map [3]. The resorption process was performed by an

iterative algorithm that has two main steps: (a) colonization of a pore by resorbing cells, (b) resorption of the substitute material. The ceramic volume fraction (CVF) was calculated after iteratively. Experimental [2] and theoretical data were compared. Prony expansion was used to fit exponential functions to resorption curve (Eq. 1):

$$f(t) = \sum k_i \, e^{Ai\,t} \qquad i=1, 2, 3 \quad \text{(Eq. 1)}$$

Where $f(t)$ is the CVF, t represents time, K_i and A_i are constant and rate coefficients, respectively. Resorption rate was estimated using the derivative of $f(t)$ (Fig. 1).

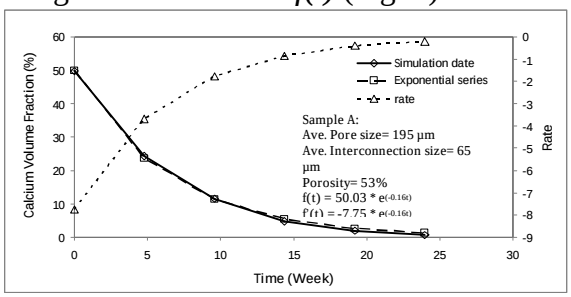

***Fig.1**: Exponential series approximation of the simulation results and resorption rate data*

In addition, each scaffold was divided into 3 cylindrical zones with 3 increasing radius. The rate was calculated for different zones.

RESULTS

Table 1 summarizes resorption rate of the four scaffolds. The resorption rate reduced with decreasing the CVF over time. For equal value of ceramics, the resorption rate was faster for group B compared to groups. The slow resorption rate of group D with big pore size can be attributed to less surface area available for resorbing cells. [3]

Substitute	Pore size (µm)	Resorption rate		
		CVF= 30%	CVF= 20%	CVF= 10%
A	128	4.65	3.10	1.55
B	195	5.03	3.35	1.68
C	364	4.45	2.97	1.48
D	871	4.11	2.74	1.37

***Table1**. Resorption rate at different remaining CVF volume*

Figure 2 compares the resorption rate of different zones of substitute. Local resorption analysis showed faster resorption at the outer area of samples with small pore size, samples A and B. Samples of large pore and interconnection size such D did not show notable difference.

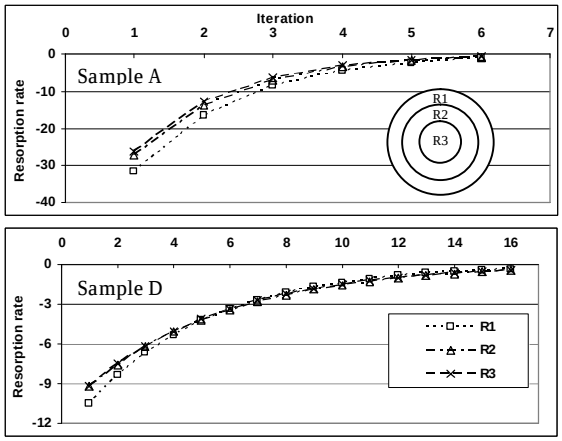

***Fig.** 2: Resorption rate of substitutes at different zones.*

CONCLUSION

The new algorithm helps understand the biological results. With this strengthening tool, we can determine resportion rate. The results for the fastest resporting substitute and that resoprtion is stronger on the outside are consistent with the biological results.

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CHARACTERIZATION OF POROUS CALCIUM PHOSPHATE SUBSTITUTES USING MICRO-COMPUTED TOMOGRAPHY AND FUZZY IMAGE PROCESSING METHODS

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INTRODUCTION

The 3D structure of calcium phosphate (CaP) bone substitutes plays important role in new bone formation and the resorption. Designing optimal substitute requires accurate characterization of the scaffold structure. Micro-computed tomography (μCT) provides access to 3D structure and allows for more detail geometric analysis. Several methods have been developed to quantify 3D structure of bone substitute based on μCT images. In this study, we describe a fuzzy image processing method to estimate the structural parameters such as porosity, pore size and interconnection size of β–TCP scaffolds.

METHODS

The cylindrical samples of β-TCP substitutes were fabricated by emulsion method at different synthesis conditions (sintering temperature of 1000°C, 1200°C and 1350°C, and Ca/P molar ratio of 1.45, 1.475 and 1.5) [1]. The samples were scanned by μCT system with 15 μm resolution. For geometric analysis, the scanned images were fuzzified by sigmoidal function. The porosity was calculated based on fuzzified images. The fuzzy distance transform (FDT) map was then determined for each voxel of pore space. This value is defined as the shortest fuzzy distance between that voxel and the background. Geometrical parameters were quantified by the max-min detection algorithm which found the local maxima and saddle voxels of FDT map [2]. The average pore size and interconnection size are equal to the average FDT values of local maxima and saddle voxels, respectively. Particularly, the average pore size is obtained using two definitions: arithmetic average values (number-based) and volume weighted average value (volume-based) [2].

RESULTS

Fig. 1 shows the pore size distributions of samples as a function of (a) Ca/P molar ratio and (b) sintering temperature. As illustrated in Fig 1, synthesis condition did not considerably affect on the distribution of pores. Geometric analysis also exhibited no significant effect of synthesis parameters on geometrical measurements. Interconnection size varied between 36 and 46 μm. Average pore size was in the range of 207-272 μm (number-based) and 268-373 μm (volume-based), respectively.

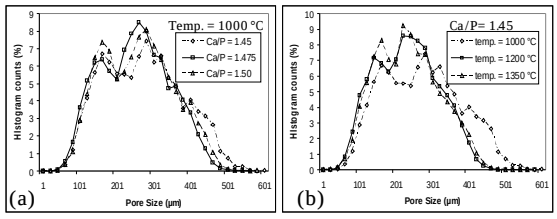

***Fig.1**: Pore size distributions as a function of (a) Ca/P molar ratio and (b) sintering temperature*

Figure 2 shows local porosities along the longitudinal axis of the scaffold (variability < 1.8%). The small variability and deviations (Fig. 2) between the local porosity values are evidence of homogeneity of scaffold internal structure.

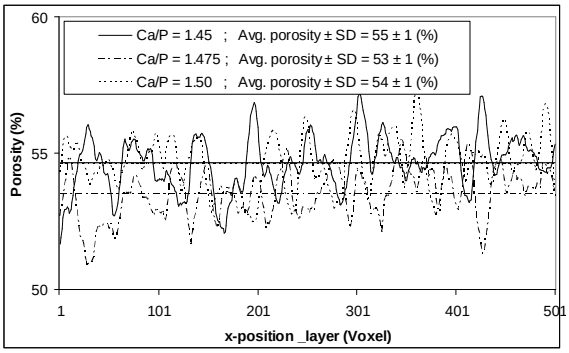

***Fig.2**: Local porosity versus x-position of layers; samples were produced at sintering tem. of 1000 °C.*

CONCLUSION

μCT and fuzzy image processing tools have been integrated for accurate quantification of the 3D scaffold structure. These methods also provide information to study the effect of structural parameters on bone formation and scaffold resorption.

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INJECTABLE APATITE / BLOOD COMPOSITE: DEVELOPMENT AND CHARACTERIZATION

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INTRODUCTION

Apatitic calcium phosphate cements (CPCs) are used as bone substitutes in reconstruction surgery because of their similarity to bone mineral and bioactive properties. Conventional CPCs are stiff and fragile materials and most of them do not present any porosity able to stimulate volume cell colonization [1-2]. These drawbacks limit surgical use of CPCs to small volume and non load bearing applications. In order to decrease its stiffness, fibrin, obtained from blood clot, has been considered as a resorbable polymer that could be added to an apatitic CPC formulation. The purpose of this study was comparing both mechanical properties and porous structure of a given CPC formulation with another one where the liquid component was replaced by fresh blood.

MATERIALS AND METHODS

Two different CPC formulations were studied. They were all containing a solid component made of : □-TCP [$Ca_3(PO_4)_2$], DCPD [$CaHPO_4,2H_2O$], CDA [$Ca_{10-x}[\ ]_x(HPO_4)y(PO_4)_{6-y}(OH)_{2-z}[\ ]_z$], MCPM [$Ca(H_2PO_4)_2.H_2O$] and a polysaccharide used to improve injectability. Liquid component was either a 5% Na_2HPO_4 solution or fresh sheep blood. In the two materials, the liquid to powder ratio was equal to 0.5. Cylinders of 12mm height and 6mm diameter - were prepared and soaked during 72 hours in saline solution at 37°C prior to determination of the compressive strength. Sample porosity was quantified on SEM micrographs (backscattered mode) using an image analysis software and complete porosity was obtained by mercury penetration. The chemical transformation of □-TCP to apatite was followed using X-ray diffraction and infrared spectrometry.

RESULTS AND DISCUSSION

At 72h after setting reaction, □-TCP was completely transformed to poorly crystalline apatite in all tested samples. At that time blood/CPC composite showed a significantly higher macroporosity rate (>200%) than conventional CPC samples. Logically, compressive strength was roughly divided by two from 12.0 MPa to 6.1 MPa respectively (N=20). Furthermore the slopes measured in the elastic region of the two curves presented on the figure showed a significant decrease (2.1) of the blood/CPC composite stiffness compared with the conventional cement. A more plastic behaviour was also observed for the composite.

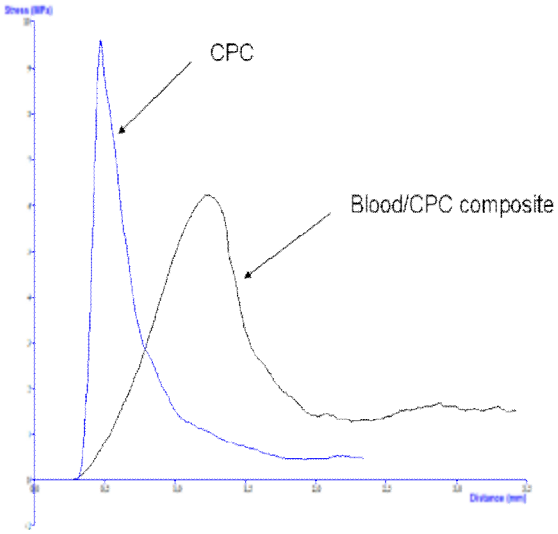

Figure: Comparative compressive strength tests for CPC and blood/CPC composite.

This study showed that blood/CPC composites present interesting mechanical features and a significant induced macroporosity that are required for bone substitution. Furthermore, it is known that hematoma formation plays a central role in the first step of healing through the formation of a clot composed of fibrin matrix, serum and blood cells. Therefore our next study will investigate the specific in vivo bioactivity of autologous blood/CPC composites.

ACKNOWLEDGMENTS

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THE USE OF CALCIUM CARBONATE CEMENTS AS A DRUG CARRIER

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INTRODUCTION

Several pathologies, such as cancer and chronic pain,would benefit from localized and/or prolonged drug release [1-3]. This could be delivered through a minimally invasive surgical procedure using an injectable, resorbable cement carrying the drug of choice [3]. Whereas one such cement is already in use in the treatment of prostate cancer [3], cements with a lower resorption rate may be of interest due to a wider range of applications. In this work, a feasibility study was performed with respect to the drug elution capabilities of a calcium carbonate cement.

MATERIALS AND METHODS

Calcium carbonate (CaCO3) was synthesized in the form of aragonite powder. Calcium sulphate (20%) was added to the powder in order to reduce the setting time of the cement. A model drug, Theophylline, was also added in amounts of 5 and 50mg. A cement paste was created by the addition of water (liquid/powder=0.5) and cylindrical specimens (8mm diameter, 3mm high) were produced. The specimens were left to harden at 37ºC for 24h. The setting time was measured using Gillmore needles and the chemical and structural composition were evaluated using X-ray diffraction (XRD) and SEM analysis. The drug elution was measured using a spectrophotometer for a buffer (pH=6.8) and an acid solution (pH=1), representing a common *in vivo* situation as well as the more acidic stomach environment.

RESULTS

The XRD analysis of the set cement showed a decrease in the Theophylline peaks and a slight increase for the calcite peaks compared to the original powder phase. SEM analysis confirmed that the main component of the cements appeared to be agglomerated aragonite particles, rather than transformed calcite, which is the thermodynamically stable phase of CaCO3 [4]. The cement containing more model drug was found to have a shorter setting time (2-2.5h compared to 3-3.5h). This may be due to the slight acidity of the drug facilitating the dissolution and re-precipitation of the carbonate.

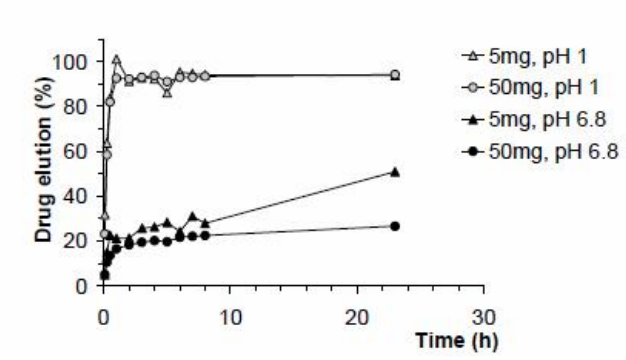

Fig.1. Elution curves for cements containing 5 and 50mg of the drug in an acid solution (pH=1) and a buffer solution (pH=6.8)

The acidic pH of 1 gave rise to a complete elution of the drug in a very short time (Figure 1), due to the high dissolution rate of the cement at this pH. At a higher pH, a slower release profile was found (Figure 1). However, there was a substantial elution of the drug despite the samples retaining their shape throughout the testing, suggesting that the cement erosion was not a requisite for the drug release. Furthermore, the elution data did not give a linear relationship between the drug released per unit surface area and the square-root of time (i.e. Higuchi's law), suggesting that diffusion alone was not the rate limiting factor either. This implies that the drug elution from this calcium carbonate cement is governed by complex interactions and further studies are needed to clarify the roles of the different components as well as other factors such as amount and distribution of cement porosity.

CONCLUSION

The feasibility of using a calcium carbonate cement as a resorbable drug carrier was investigated in this study. The drug elution process was found to be subject to complex interactions and further studies are necessary to clarify the elution mechanism.

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CLASSIFICATION OF INJECTABLE BIOACTIVE COMPOSITE FOR BONE REPAIR

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Injectable CaP biomaterials should associate efficient bone colonization on implantation with non-invasive surgical techniques. Two types of injectable bone substitutes (IBS), are being developed in laboratories: calcium phosphate cement and calcium phosphate suspensions. The concept of apatitic calcium phosphate cement (CPC) was first introduced by LeGeros in 1982 [1]. The first patent on hydraulic CPC cement (self-setting or self-hardening) was obtained by Brown and Chow in 1988 [2]. In the 1990s, considerable efforts were made to develop injectable bone substitutes. Self-setting Calcium Phosphate Cements (CPC) were the first injectable bone substitute developed for percutaneous applications [3]. The calcium phosphate cements were first used as bone substitutes to repair cranio-facial defects. The histological observations on animal model [4, 5] showed a bone-implant interface with proliferation of osseous cells in the volume of the implant after several weeks, and then a slow reduction in the biomaterial with a new bone formation after several months. These hydraulic cements, however, are not ready-to-use, requiring extemporaneous mixing with various in situ setting times. Furthermore, most CPC wash out when they come into contact with body fluids prior to setting [6]. Additionally, once hardened, CPC produce

a dense material with irregular microporosity and slowly degrade in vivo [7, 8], whereas numerous studies have shown that interconnected macropores are needed to facilitate bone ingrowth [9]. A second type of injectable bone substitute, consisting of CaP ceramic granules suspended in a water-soluble polymer carrier phase, has been developed.

For this paper we propose a classification for injectable bone substitutes:

As for all classifications, it is not easy to compile a classification of the entire injectable bioactive composite for bone repair. The first classification can be the chemistry of the compound used to produce these materials. Like the definition of composites, all these materials are associations of different materials and it could be difficult to characterize all of them. The only easy way to differentiate them is the chemistry of the mineral phase being used and which can be calcium sulfate, calcium phosphate like brushite [$CaHPO_4 2H_20$], apatite [$Ca_5(PO_4)OH$], hydroxyapatite HA, beta tricalcium phosphate βTCP [β-$Ca_3(PO_4)_2$], a biphasic calcium phosphate which is an association of HA and βTCP, etc. These calcium phosphates can be the result of a cement process which is the terminal compound of an acid-base reaction in water. There are two main types of hydraulic calcium phosphate cements (CPC), depending on the end product of the setting reaction, brushite and apatite CPC. Calcium phosphate granules or fillers can be synthetic-like sintered ceramics after grinding or fillers of biological origin like bovine or porcine bone.

The mechanical properties can decipher two types of IBS: the first is the hardening IBS and the second is non-hardening and is composed of calcium-phosphate suspension. The last classifications can be the presentation, ready to use or not. The question is whether the porosity is interconnected or not. And can we use cells with IBS for bone tissue engineering?

One way to present IBS is via its physical presentation to the host bone and cells. This classification is very simple and can explain the kinetics of bone ingrowth which is at the origin of the biological and mechanical properties, and is more relevant than the initial mechanical properties.

The first physical presentation, Class I, is particulate surfaces of calcium phosphate into a fast resorbable matrix which disappear quickly, allowing fast osteoconduction from the wall of the bone cavity to the center. The fillers have slow resorption kinetics and act as the scaffold for the bone ingrowth. The main advantage of this presentation is the fully interconnected properties [10] of the composite after the matrix dissolution and/or degradation.

In this Class I, IBS, we possess (i) non-hardening and (ii) hardening materials. For the hardening materials the matrix can be organic or mineral. The disadvantage is the lack of initial mechanical properties.

The second physical presentation, Class II, is a dense calcium phosphate matrix associated with particulate porogens. These porogens will disappear after dissolution or/and degradation that give controlled porosity of the IBS to increase the kinetics of bone ingrowth and substitution. The greatest favor advantage of this presentation is the initial mechanical properties but the disadvantage is slow kinetic of bone ingrowth depending on macroporosity interconnection.

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IN VIVO EVALUATION OF COTTON WOOL-LIKE AMORPHOUS-TCP/PLGA NANOCOMPOSITES

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INTRODUCTION

In reconstructive surgery, bioresorbable implant materials are in great demand for the repair of bone defects. Many current biomaterials consist of calcium phosphates which excel in biocompatibility, bioactivity and osteoconductivity. However, many commercially available products are limited to specific clinical applications because of their brittleness, incompressibility and difficulty to shape. The present contribution shows two in vivo studies of entirely in vitro [1,2] investigated nanocomposites.

METHODS

Highly porous nanocomposites consisting of a biodegradable poly (lactide-co-glycolide) fibrous matrix and either aerosol-derived amorphous tricalcium phosphate nanoparticles (PLGA/TCP) alone or finely silver dispersed on identical TCP nanoparticles (PLGA/Ag-TCP) were prepared by electrospinning. A first in vivo study was performed in four circular non-critical size calvarial defects in New Zealand White rabbits, which were treated with PLGA and PLGA/TCP scaffolds (Fig. 1a). The “gold standard” in dental surgery (BioOss®) was used as a positive control and cavities left empty served as a negative control. The bone regeneration was assessed after 4 weeks implantation using histological and micro-computed tomographic analysis. The in vivo performance of PLGA/TCP and PLGA/Ag-TCP scaffolds was evaluated in a second study using a drill hole defect model in the metaphysis of long bone in sheep (during 8 weeks). The effect of silver on the biocompatibility and cellular reactions during bone regeneration was assessed by histological analysis applying a score system.

RESULTS

The fibrous nanocomposites showed enhanced in vitro mineralization in simulated body fluid (Fig. 1a), whereas silver containing fibres showed additionally strongly antimicrobial properties for 2 days against E. coli [2]. The cotton wool-like biomaterials could be applied very easy during surgical procedure. The area fraction of newly formed bone was significantly increased for TCP containing fibres compared to pure PLGA [3]. Semi-quantitative histology showed that both PLGA/TCP and PLGA/Ag-TCP scaffolds were fully biocompatible and enabled fast bone formation even to the centre of the former defect (Fig. 1c). No inflammatory reactions were observed for both biomaterials which were mostly resorbed through macrophages (Fig. 1d).

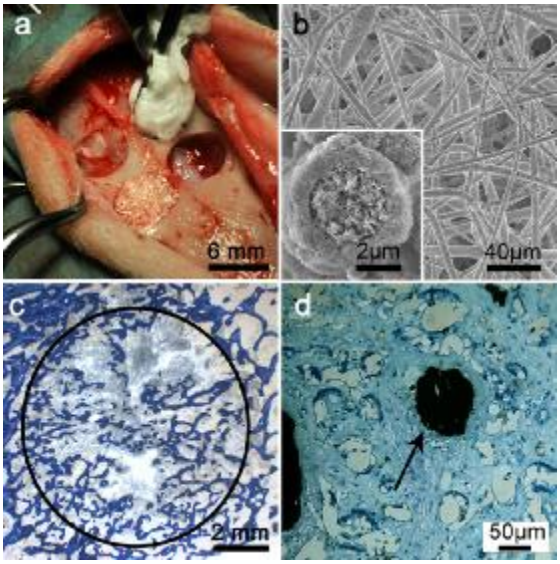

Fig.1:(a) The Cotton wool-like biomaterial is easy-to-apply in bone defects. (b) SEM of PLGA/ TCP nanocomposites after in vitro mineralization. (c, d) Histological sections showing bone formation and resorption of material in sheep.

DISCUSSION & CONCLUSIONS

The easy applicable biomaterial suggests application for non-load bearing complex shaped bone defects. The advantageous use of this compressible material could be valuable in minimally invasive surgery or for specific indications in dental surgery (i.e. sinus augmentation, augmentation procedures of the alveolar bone, socket preservation after tooth extraction) where the antimicrobial properties of PLGA/Ag-TCP could be beneficial.

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SILICON DOPED CALCIUM PHOSPHATE CEMENT: PHYSICO CHEMICAL PROPERTIES AND IN VITRO CELL RESPONSE

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INTRODUCTION

The interest of obtaining silicon doped calcium phosphates arises from the condition of silicon as one of the trace metals associated with bone metabolic processes, mainly with its development during the first stages [1]. In this context, numerous works suggesting an increase in the bioactivity of Si-subtituted calcium phosphates have been published in recent years [2, 3]. Moreover, silicon is known to have a stabilising effect of the alpha polymorph of tricalcium phosphate (TCP, Ca3(PO4)2) [2,4]. In this work, the preparation of a calcium phosphate cement doped with silicon is proposed, using a Si-stabilized TCP as a reactant. The properties and the in vitro cell response to the ions released by the cement are evaluated.

MATERIALS AND METHODS

A calcium deficient hydroxyapatite free of magnesium ions (CDHA, CalbioChem) and an aqueous suspension of nanometric SiO2 (Cab-O-Sperse) were mixed with water and milled to ensure the intimate mixture of the reactants. The resulting paste was dried and sintered at 1250ºC for 2h, followed by a slow cooling in the furnace. No quenching was applied. The effect of silicon content on the stabilisation of the alpha form of TCP was analysed by testing different ratios CDHA:SiO2. The optimal conditions were selected to prepare silicon doped TCP (Si-α-TCP). The crystalline phases formed and the presence of any amorphous phase were

evaluated by X ray diffraction (XRD) using the external standard method. The Rietveld method was used to quantitatively refine the results obtained. A silicon-doped calcium phosphate cement (Si-CPC) was prepared by mixing the Si-α-TCP powder with water. Cylindrical moulds were filled with the paste and immersed in Ringer's solution. The same procedure was followed to prepare a control CPC, using an α-TCP powder free of silicon in this case, which preparation consisted on heating the CDHA to 1450ºC for 2h followed by quenching in air.

The transformation of the cement phases was evaluated by XRD. The set cements were characterized in terms of compressive strength, microstructure evolution (SEM) and specific surface area (N2 adsorption). A cell culture study was performed in order to evaluate the effect of the ions released by the cement on cell proliferation and differentiation. To this end, Human osteoblastic-like SAOS-2 cells were cultured in inserts that were introduced in well plates containing preset CPC and Si-CPC discs in order to allow the cells being in contact with the media containing the ions released from the different cements. Cell proliferation and differentiation were evaluated at 6h, 3,7,14 and 21 days, by LDH and ALP activity respectively. In parallel, the P, Ca and Si ion concentrations on the supernatant medium at the same time points were analyzed by ICP-OES.

RESULTS

Wet milling of CDHA with SiO2 followed by sintering proved to be an effective method to stabilize the α-TCP structure. Quench was unnecessary to obtain α-TCP as the single crystalline phase, the amount of this phase increasing with SiO2 content. A significant amount of an amorphous phase, close to 10wt%, was also detected due to the presence of silicon. The setting and hardening properties, microstructure and specific surface area of the silicon-doped cements were very similar to those of the undoped CPC, transforming in both cases into a calcium deficient hydroxyapatite after 1 day.

The ion release analysis showed a similar and sustained calcium depletion in the cell culture media where the two kinds of cements were immersed. The phosphorous concentration was similar for the supernatants of both cements, slightly higher than in the control medium, and the silicon released was around 50ppm for the Si-CPC. Cell proliferation was reduced in similar proportion when the cells were cultured in the inserts in contact both with the CPC and the Si-TCP (Figure 1). In contrast, cell differentiation was strongly enhanced in both series as compared to the control medium (Figure 2). ALP activity was significantly higher in the Si-CPC than in the CPC at 14 days, suggesting an enhancement of osteoblast differentiation by the silicon species in solution as pointed out by some studies [5].

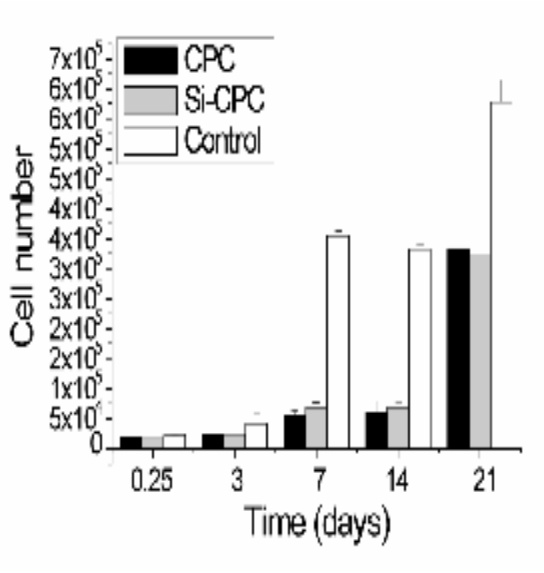

Figure 1. Cell proliferation quantified by LDH

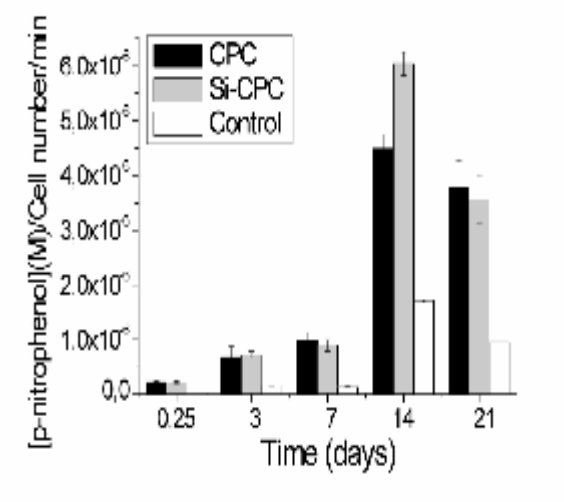

Figure 2. Cell differentiation measured by ALP activity

CONCLUSION

A single crystalline phase Si-stabilised α-TCP was obtained by a very simple method, avoiding quenching to retain the α-TCP structure to room temperature. The silicon-doped cements fulfilled the basic clinical requirements. The ions released from both cements reduced osteoblastic-like cell proliferation and enhanced cell differentiation. A further enhancement of cell differentiation was detected in the silicon-doped CPC.

ACKNOWLEDGMENTS

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INFLUENCE OF SINTERING TEMPERATURE, SINTERING TYPE AND CA/P RATIO ON INJECTABILITY OF CAP-BASED PASTES

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INTRODUCTION

Injectability of suspensions depends among others on particle size, particle size distribution, viscosity of liquid phase, liquid-to-powder ratio (LPR) and particles interactions. The aim of this study was to assess the potential of a reduction of the mean particle size on the injectability of a suspension. The reduction of particle size was achieved by choosing low sintering temperature. An improvement of injectability with reduction of the particles size was expected [1, 2]. Indeed, reducing the mean particle size decreases the size of the interstices between the particles. This should prevent the flow of the liquid in the interstices between the particles, and thus should prevent phase separation or filter-pressing, improving injectability.

MATERIALS AND METHODS

Fine β-TCP powders were prepared using hydroxyapatite (HA) – dicalcium phosphate (DCP) reactive sintering, according to equation 1.

$Ca_5(PO_4)_3OH + CaHPO_4 = 2Ca_3(PO_4)_2 + H_2O$ Eq. 1

The raw materials were HA (Budenheim, Tri-Cafos PF/C53-81, MV 2497, Lot. No. A99068A) and DCP (GFS, Art. No. 1548, Lot No. P781362, Columbus OH, USA) powders. Appropriate amount for Ca/P ratios of 1.50 or 1.475 were mixed for 10 min in a Turbula with ZrO_2 beads. After mixing both batch of powder were calcined for 12h at 850°C, then split into 4 equal parts. All 8 parts were milled with water for 15 min in a planetary mill with ZrO_2

beads. The powders were then dried and, except for 2 samples (1.1 and 2.1), calcined again at 850, 900 or 950°C. Powders 1.1 and 2.1 will be referred to as 1-step sintered powders, the other ones as 2-step sintered powders. A commercial β-TCP powder (Fluka, Art. No. 21218, Lot No. 1338057, Buchs, Switzerland) was also used as a model powder [1, 3, 4].

The powders were then characterized: Particle size and appearance of the powders were observed by SEM, specific surface area (SSA) was measured using N2 adsorption and applying the BET model and XRD measurement were performed to get the crystalline composition with Rietveld refinement. The plastic limit (PL) of the powders was as well assessed.

Pastes were prepared by mixing each powder with deionised water in a liquid-to-powder ratio (LPR) of 0.40ml/g. The powder and the liquid were mixed manually in a plastic beaker with a spatula. After a resting time of 1 day, the pastes were mixed again with a spatula and inserted into 1ml syringes (BD, Art. No. 301283, Lot No. 9106387, Franklin Lakes, NJ,USA). The injection was tested through needles with inner diameter of about 0.21mm (0.4x1.6mm, Terumo, Art. No. NN-2716R, Leuven, Belgium).

For the injection tests the syringes were held in a steel cylinder and compression force was applied by a compression test machine (Zwick 1475 UPM, Software: TestXpert V 11.0) with a displacement speed of 0.4mm/s. Three replica were tested for each powder.

The results were statistically analysed using ANOVA. A regression model considering the linear effects of the Ca/P ratio and sintering type (two-level factors), and the linear and quadratic effects and interactions associated with the sintering temperature (three-level factor) was used. Adjusted values were calculated according to the adequate statistical model, taking into account the regression parameters found significant at the (1-p) confidence level (p=0.01). The results were divided in two factorial designs: 22x3 for analysis of Ca/P ratio and sintering type and 2x3x3 for analysis of Ca/P ratio and sintering temperature. Comparison with the Fluka β-TCP powder were done with t-tests (p=0.05).

RESULTS AND DISCUSSION

The XRD analysis revealed that all powders contained HA, but no Ca2P2O7. The amount of HA as determined by Rietveld refinement ranged from 2 wt-% up to 16 wt-% (**Table 1**). One-step sintering led to higher HA content than 2-step sintering and using a Ca/P ratio of 1.475 instead of 1.5 decreased HA content. The lowest HA content (2 wt-%) was found in powders sintered in 2 steps and with Ca/P=1.5. According to ASTM F1088-87, β-TCP for surgical implantation must have a minimum purity of 95%; only powders sintered in 2 steps and with Ca/P=1.5 met this requirement. The crystallite size of each phase was calculated from isotropic peak broadening using the Scherrer formula (**Table 1**). No systematic change of the crystallite size was observed with a change of the synthesis conditions. SSA varied between synthesis procedures, but without correlation with sintering temperature, Ca/P ratio or sintering step (**Table 1**). All in-house produced β-TCP powders had much higher SSA than the commercial β-TCP from Fluka. No significant difference was found between plastic limits of all powders (**Table 1**), including Fluka powder, but this might be due to the reduced number of measurements.

The SEM pictures revealed a tendency to increase particles size with sintering temperature and sintering step (**Figure 1**). Contrary to what was expected, a 2-step sintering did not appear to reduce the particle size. Increasing the Ca/P ratio decreased the particle size.

Increasing the Ca/P ratio significantly improved the injectability of the pastes (**Table 2 and Table 3**). The statistical analysis also revealed that the sintering

temperature did not affect the paste injectability (**Table 3**). Contrary to what was expected, a 2-step sintering significantly decreased the injectability compared to 1-step sintering (**Table 2**).

Fluka β-TCP powder was 31±2% injectable, which is significantly (t-test, $p<0.05$) lower than powders produced with a Ca/P ratio of 1.5 and powders with a one-step sintering.

Table 1 Summary of characterisation results.

SINTERING TYPE	CA/P	TSINT [°C]	**B**-TCP [%] *	HA [%] *	CRYSTAL. SIZE **B**-TCP[NM]*	CRYSTAL. SIZE HA [NM] *	SSA [M2/G] (N=3)		PLASTIC LIMIT [ML/G] (N=2)	
1-STEP	1.475	850	91	9	75	50	6.5	± 0.0	0.31	± 0.00
2-STEP	1.475	850	98	2	99	31	6.8	± 0.1	0.26	± 0.01
2-STEP	1.475	900	98	2	88	30	7.9	± 0.1	0.29	± 0.01
2-STEP	1.475	950	98	2	107	33	5.8	± 0.6	0.28	± 0.01
1-STEP	1.5	850	84	16	77	52	7.7	± 1.0	0.29	± 0.00
2-STEP	1.5	850	92	8	76	47	6.2	± 0.1	0.28	± 0.02
2-STEP	1.5	900	92	8	101	55	5.9	± 0.1	0.29	± 0.01
2-STEP	1.5	950	93	7	84	54	5.8	± 0.2	0.29	± 0.02
FLUKA **B**-TCP							0.9	± 0.1	0.30	± 0.01

*N=1

Table 2 Injectability in function of Ca/P ratio and sintering type for sintering temperature of 850°C. 22x3 factorial design.

Ca/P [-]	sintering type	Inj [%]	Inj(Adj) [%]	± 3.25 stand. error
1.475	1-step	55%	57%	11%
1.475	2-step	21%	19%	11%
1.5	1-step	84%	82%	11%
1.5	2-step	44%	45%	11%

Table 3 Injectability in function of Ca/P ratio and sintering temperature for 2 step-sintering. Sintering temperature has no significant influence. 2x3x3 factorial design.

Ca/P [-]	sintering T [°C]	Inj [%]	Inj(Adj) [%]	±2.98 stand. error
1.475	850	21%	27%	17%
1.475	900	17%	27%	17%
1.475	950	43%	27%	17%
1.5	850	44%	50%	17%
1.5	900	55%	50%	17%
1.5	950	52%	50%	17%

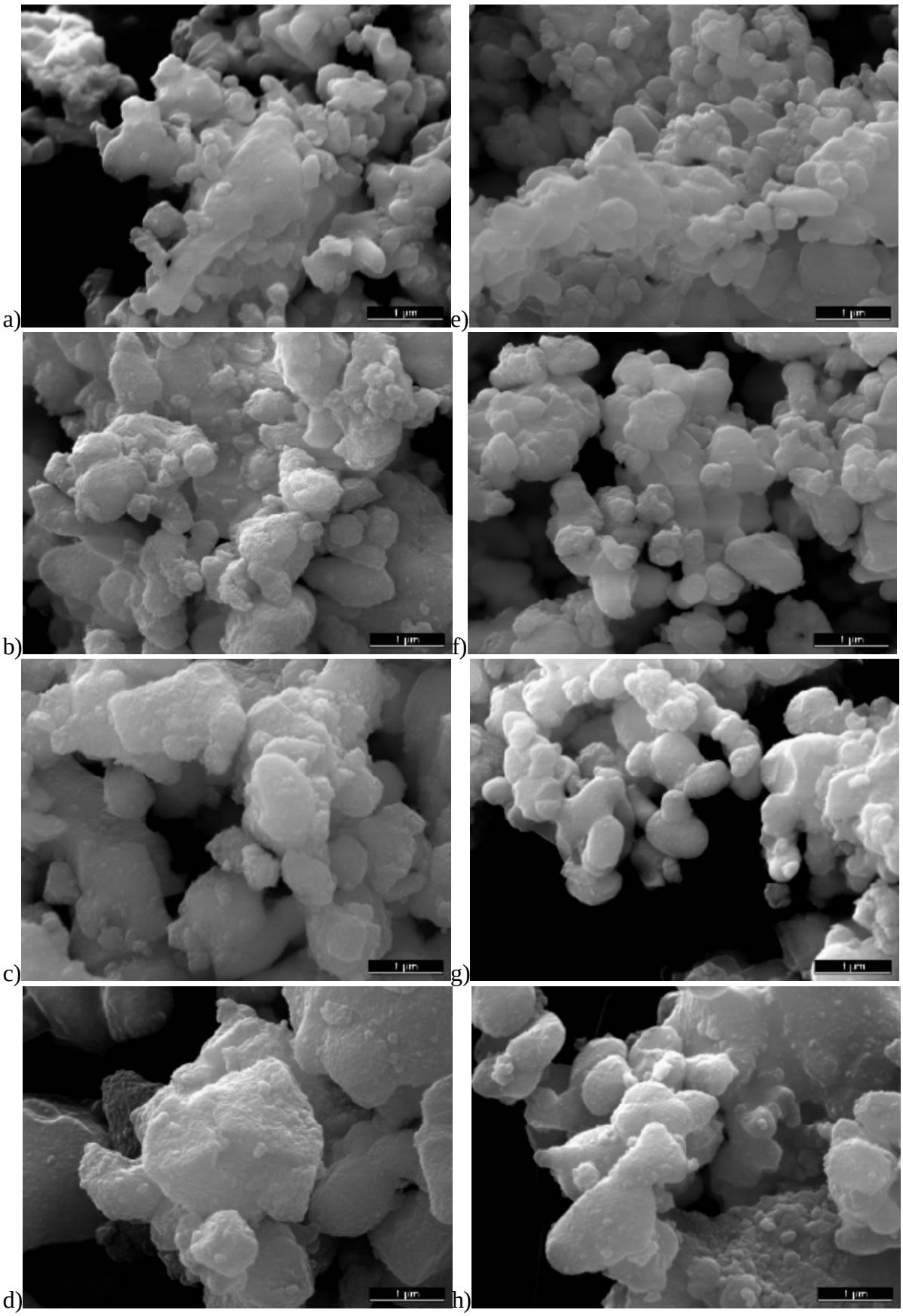

Figure1 SEM pictures (20’000x):
a) 1-step, 850°C, Ca/P=1.475;
b) 2-step, 850°C, Ca/P=1.475;

c) 2-step, 900°C, Ca/P=1.475;
d) 2-step, 950°C, Ca/P=1.475;
e) 1-step, 850°C, Ca/P=1.5;
f) 2-step, 850°C, Ca/P=1.5;
g) 2-step, 900°C, Ca/P=1.5;
h) 2-step, 950°C, Ca/P=1.5.

CONCLUSION

As expected, decreasing the sintering temperature clearly decreased the particles size, as observed on SEM pictures, but surprisingly this had no influence on injectability. Increasing the Ca/P ratio reduced the particles size and significantly improved the injectability. Contrary to what was expected, the use of a two-step sintering seemed to slightly increase the particle size and decreased injectability. No correlation was found between SSA and injectability.

ACKNOWLEDGMENTS

The results presented in this study were partly generated in the frame of a project supported by CTI 9170.1, Delta Robotics GmbH.

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LIGHT-CURABLE BIOACTIVE POLYMERIC COMPOSITE GLUES FOR BONE DEFECT TREATMENT

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INTRODUCTION

Recent research on bone implant materials targets combination of resorption and sufficient mechanical properties [1]. Photopolymerizable acrylic cements [2] possess high mechanical properties and show good biocompatibility. However a major drawback is the difficulty to set them in vivo. Self-setting tricalcium phosphate (TCP) cements have high resorption and fast setting time, though they have low mechanical strength [3]. The current work represents Heliobond® adhesive, consisting of bisphenol A diglycidyl methacrylate and Tri (ethylene glycol) dimethacrylate (BisGMA/TEGDMA), used as a matrix and nanosized TCP as a filler composites. So far, this study describes the effects of embedding amorphous TCP nanoparticles in light-curable resin (Heliobond®) and its influence on bioactivity and bone adhesion strength.

MATERIALS AND METHODS

Amorphous TCP nanoparticles were prepared by flame spray synthesis [4]. The mixtures of polymer and TCP nanoparticles were used as precursors for composite and cured by blue light (λ = 450 nm) in different time periods (15 to 30 seconds). The polymeric resin was loaded with 0 and 20 wt. % TCP fillers. Composites were mixed manually with a spatula and afterwards were molded in forms of platelets (10x80x0.5 mm) for in vitro degradation tests and cylinders (9x4.5 mm) for mechanical testing. Samples were immersed in simulated body fluid (SBF) solution at 37 °C for 1, 3, 7 and 14 days.

The adhesion of pure polymer and Heliobond®/TCP composite on cow hip bone surface was designed to maintain "close to in vivo" conditions (Fig. 1).

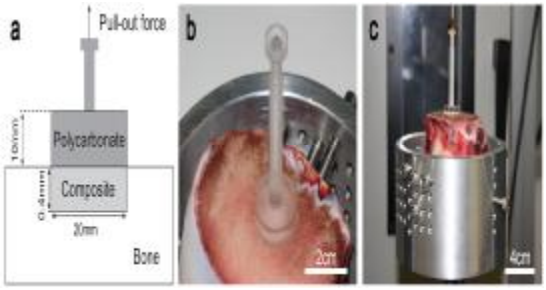

Fig.1: Adhesion to bone: (a) Schematic test setup. (b) Image of a glued polycarbonate rod on a fresh cut cow hip bone after light-curing of the sample. (c) Pull-out test in a conventional testing machine.

RESULTS

In vitro bioactivity tests of pure adhesive polymer and 20 wt. % containing TCP composites showed both degradation and hydroxyapatite deposition. X-ray analysis of Heliobond®/TCP composite after storing in SBF solution for 14 days showed formation of hydroxyapatite (HAp) layer (Fig. 2a).

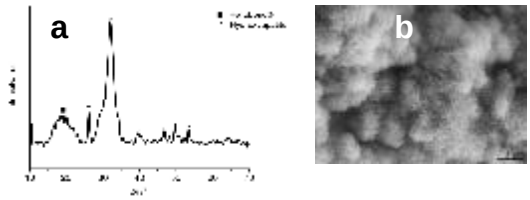

Fig. 2: (a) XRD of Heliobond®/TCP after 14 days setting in SBF. (b) SEM of sample Heliobond®/TCP after 14 days in SBF.

Scanning electron microscopy images (Fig. 2b) show deposition of needle-shaped HAp crystals formed on the surface of composite scaffolds.

Pure polymeric composites resulted in a mass loss of 7 wt. % after two weeks immersion in SBF (Fig. 3a). By embedding TCP nanoparticles in the polymer matrix, the water uptake could be increased (Fig. 3b), the shrinkage reduced and therefore these factors improved mechanical reliability.

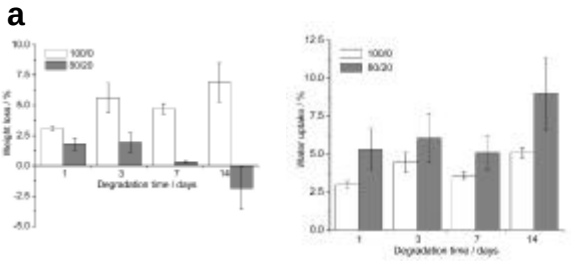

Fig.3: (a) Weight loss and water uptake (b) of different Heliobond®/TCP composites as a function of degradation time in simulated body flight.

Compressive strength measurements demonstrated stiffness and elasticity of the material comparable to natural bone. Incorporation of 20 % wt. reactive TCP nanoparticles improved adhesion to bone in close to in vivo conditions.

CONCLUSION

BisGMA/TEGDMA polymeric composites filled with TCP nanoparticles showed rapid in vitro biomineralization after immersion in simulated body fluid, high mechanical properties and enhanced adhesion to bone tissue, which could potentially apply them as in vivo light-curing biomaterials [5].

ACKNOWLEDGEMENTS

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INVESTIGATING THE EFFECT OF THE PERMEABILITY ON THE INJECTABILITY OF CALCIUM PHOSPHATE PASTES

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INTRODUCTION

There are two physical phenomena competing during the injection of a CPC paste – paste flow and filtering [1]. During filtration, particles are packed into the filter (cake) while the liquid seeps through the particles and filter. Darcy's law mainly governs this process and accordingly the powder-related factors as cake porosity, mean particle size, size distribution, particle-specific surface area, and sphericity are key for the filtering process [2]. The physical property, which captures most powder-related factors and can be measured is the permeability. It was also hypothesized that low permeable powder reduce the filtering and increase the injectability. This study will examine this hypothesis.

METHODS

Three different powders (β-TCP, HA and plasma-treated β-TCP powder) with different characteristics have been used in this study. Powders were characterized for particle shape, surface area, plastic limit, particle size and distribution, and specific area [Table 1]. In addition to the 3 original powders, the permeability and injectability of mixed powders were examined. Specifically, the original β-TCP powder was mixed with 0.5, 1, 1.5, 2 and 3% of HA powder by weight. Likewise, β-TCP powder was mixed with 1, 2, 3 and 10 % of plasma treated powder. The permeability of all mixtures was investigated at porosities values ranging from 0.645 to 0.785 according to ASTM designation C: 204 known as Blain method. The Blain apparatus has been instrumented to insure accurate measurements by installing two optical sensors on the upper and lower liquid levels to detect the manometer liquid presence inside the manometer tube. There were 9 repeats for each combination at every single porosity which ends up with a total of 324 repeats. The permeability results were then correlated to the injectability of the powder combinations.

RESULTS

There is a direct relation between the air permeability and porosity of the powder bed. The Blain times of β-TCP were 66.79(±0.96), 53.25(±0.18) and 43.39(±0.03) at 0.645, 0.66 and 0.675 porosities, respectively with 70.75 (±1.85) percentage of injectability. Adding small HA to β-TCP improved injectability reaching the highest value of 76.37 (±0.85) at 1.5 weight % HA and decreased by adding more than this percentage [Fig.1]. The same effect was noticed with the plasma treated powder.

	SPA [m²/g]	d (0.1)	d (0.5)	d (0.9)
β-TCP	5.01E+00	1.43	6.441	20.945
HA	5.04E+01	0.284	2.906	7.09
Plasma treated	9.45E+00	1.162	6.955	19.133

Table 1 Specific surface area and the particle size

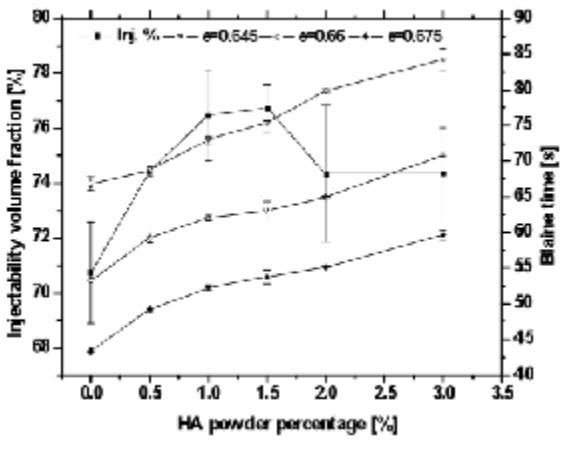

Fig.1 Effect of adding HA powder on the injectability and the Blain time of β-TCP

CONCLUSIONS

The finding on the permeability/ injectability interaction was surprising. Injectability increased by adding small

amounts of fine powders till a certain limit and then decreased. Adding fine powders in certain concentrations may achieve a critical particle size distribution to promote the flow of the paste. This effect dimishes if further fine powder was added to the original powders.

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CHARACTERISATION OF A SOUTH CHINA SEA CORALLINE HYDROXYAPATITE/CALCIUM CARBONATE AND ITS CLINICAL APPLICATION

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INTRODUCTION

Coralline hydroxyapatite (CHA) is normally produced by hydrothermal conversion from the calcium carbonate exoskeleton of sea coral calcite. We have recently developed a partially converted coralline hydroxyapatite/calcium carbonate (CHACC). CHACC contains a coral calcium carbonate scaffold that is enveloped by a thin layer of hydroxyapatite. The present study aims to characterise this CHACC by investigation of the material and biological properties in vitro, and the osteogenetic potentials both in vitro and in vivo and the clinical performance as a bone substitute following tissue resection of bone tumours in patients.

MATERIALS AND METHODS

Powder X-ray diffraction (XRD), Fourier transform infra-red (FTIR), energy dispersive X-ray spectroscopy (EDX), thermo gravimetric analysis (TGA) and scanning electron microscopy (SEM) were employed to characterize CHACC. In vitro hMSC proliferation and differentiation assays, subcutaneous implantation in vivo, and clinical observation of 16 patients after implantation were investigated.

RESULTS

The SEM, EDX, XRD and FTIR results demonstrate that the CHACC used here is a mixture of hydroxyapatite and calcium carbonate, with calcium carbonate as the main component. CHACC consists of two overlapping layers morphologically and chemically (Fig 1). CHACC supported human mesenchymal stem cell proliferation and osteogenic differentiation in vitro and in immunodeficient mice after subcutaneous implantation in vivo.
All sixteen clinical cases showed normal wound healing with no infection or other complications observed. X-ray examination showed that after implantation, visible callus formation was observed at one month; the density of the implanted CHACC decreased from three months and was accompanied by bone density increases in the gaps between the CHACC; clinical bone healing was achieved at 4 months. The majority of the implanted CHACC was degraded by 18-31 months (Fig 2).

DISCUSSION AND CONCLUSIONS

CHACC exhibits a thin layer of nanocrystalline HA on the surface. The converted coral has been shown to act as a solid-phase domain substratum for bone formation. The calcium carbonate core of CHACC is more biodegradable and shows full degradation after implantation. In conclusion, CHACC appears to be an excellent bone graft material. It biointegrates with the host, is osteoconductive, biodegradable and can be

an attractive alternative to autogenous grafts.

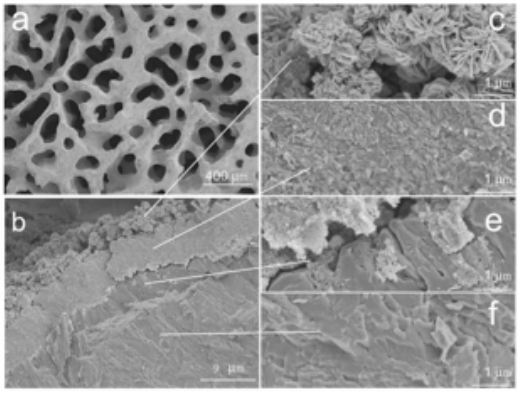

Fig 1. SEM Electronic micrographs of the surface structure of the CHACC cross-sections. (a) low magnification of CHACC. c-f shows sequential crosssections from b. (c) the surface crystalline HA, (d) a 5-10µm adjacent layer, (e) a junction between layers and (f) inner core structure.

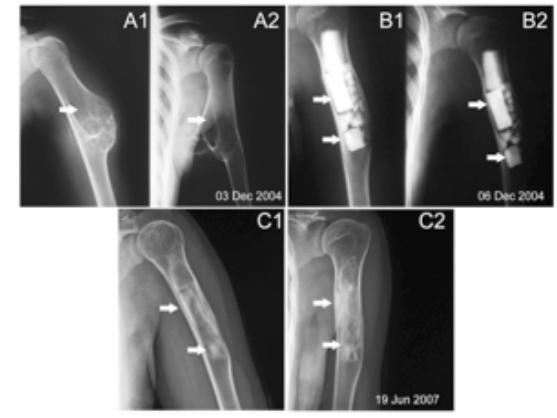

Fig 2. X-ray (anterior-1 and lateral-2) radiographs of case 1, Osteochondroma, 13 year-old female. A1-2, before operation. B1-2, after incision of the total tumor, a 9 cm × 2 cm × 2 cm cavity was formed that was filled by CHACC implantation. C1-2, two years and 7 months after operation, showing bone was completely healed, with normal function and no implications.

ACKNOWLEDGMENTS

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PRINTABILITY OF TRICALCIUM PHOSPHATE POWDER FOR BONE TISSUE ENGINEERING SCAFFOLDS

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INTRODUCTION

Scaffolds for bone tissue engineering are hierarchical porous structures aiming to attain desired mechanical function and mass transport properties like permeability and diffusion, as well as to produce these structures within arbitrary and complex 3D anatomical shapes [1]. Powder based 3D printing (3DP) is a promising solid free form technique [2] capable of generating complex cellular solids out of bioactive Tri-Calcium Phosphate (TCP) powder.
Until now the relation between the final quality of printed scaffolds and the initial powder properties is poorly understood. Flowability of powdered material is an essential requirement for the layer-based additive process of 3DP. High flowability of adequate powders allows the roller to build up thin layers and thus high 3DP resolution. This study therefore aims at better understanding of the interplay between particle size, flowability and printability for powder based 3DP.

MATERIALS AND METHODS

The β-TCP powders (Medicoat AG, CH) used in this study had an average particle size of 8µm (XS), 18µm (M), 29µm (L) and 40µm (XL). The XS powder was plasma-treated to enhance its flowability [3].
Particle size distribution (PSD) was measured by laser diffraction suspended in Ethanol (Mastersizer 2000 / Hydro 2000S, Malvern, GB). The specific surface area (SSA) was determined by BET method using N2 adsorption (Gemini 2360, Micromeritics, USA).
Powder flowability was measured with the so called Ring Shear Tester (RST-XS, Schulze Schüttgutmess-technik, DE) [4]. The ring shear cell was filled with a volume of 30ml, the pre-shear stress was set to 1500Pa, and shear stresses of 300/750/1200/300N were applied. Flowability was expressed by the flow

function coefficient (ffc; large ffc value = high flowability).

Five 89µm-thick powder layers were deposited on top of each other using a 3D printer (*cp. Fig.1, Z-Printer 310, Z-Corporation, Burlington, MA, USA*).

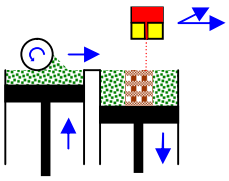

Fig. 1: Schematic principle of 3D printing

The printability was defined as the ability of the 3D printer to deposit a flat powder layer quantified by measuring layer roughness (Ra: arithmetic average roughness), which was measured by applying 3D surface reconstruction analysis (MeX V5.1, Alicona Imaging, AT) on low vacuum SEM images of the powder surface (Zeiss EVO MA 25, VPSE detector, 20kV acc. voltage, VP target 45Pa, N2 partial pressure).

Results and discussion

A typical qualitative result of the reconstructed 3D surface is depicted in Fig.2:

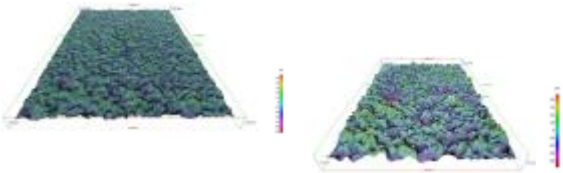

Fig. 2: Surface of TCP powder M (left) and L (right)

Table 1 summarizes the relevant quantitative results.

Powder	d10/d50/d90 [µm]	ffc [-]	Ra [µm]	SSA [m2/g]
XS	1.8/8/18	1.84 ± 0.07	19.2 ± 0.5	1.01 ± 0.01
XS Plasma	1.4/6/13	3.59 ± 0.03	16.2 ± 0.9	1.01 ± 0.01
M	11/18/28	5.34 ± 0.23	7.9 ± 0.3	0.34 ± 0.02
L	17/29/50	7.31 ± 0.18	17.5 ± 0.6	0.30 ± 0.02
XL	25/40/62	7.72 ± 0.29	27.7 ± 1.2	0.32 ± 0.01

Table 1: Percentiles of PSD, flowability, roughness and specific surface area results

These results reveal a linear correlation between the mean particle size and the flowability factor (R2=0.9). The specific surface is about 3 times higher for the XS powder than for the rest. Surface roughness seems to be optimal in the range of the M powder. Small particles tend to agglomerate, while large particles exhibit an inherent high roughness. The tendency of small particles to agglomerate can be reduced with a plasma coating by decreasing the van der Waals forces between particles and thus doubling the flowability. However, the roughness remains in a suboptimal range for 3D printing.

CONCLUSION

This investigation quantifies the 3DP relevant powder characteristics which in turn sets a basis for systematic optimization of 3DP for bone tissue engineering scaffolds.

ACKNOWLEDGEMENTS

Funding from RMS Foundation is gratefully acknowledged.

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A CALCIUM SULPHATE/ CALCIUM PHOSPHATE AS AN INJECTABLE, BIOACTIVE AND RESORBABLE BONE SUBSTITUTE

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INTRODUCTION

Mini-invasive surgery raised the needs for injectable bone substitutes. However, current injectable calcium phosphate bone

substitutes made of calcium phosphates are often difficult to inject with a syringe and exhibit a long setting time. Most of them have also a low cohesion after injection in an open surgery area and a low recolonization by the bone due to the small size of porosities and a low resorption when their final composition is Hydroxyapatite[1]. On the other hand, calcium sulfate (CaS) bone substitutes have generally a better handling behavior but a too fast resorption to allow a recolonization of the defect by the new bone[2,3]. To address these major issues, a new bone substitute was developed in this study, using a composite of hydroxyapatite and calcium sulfate.

EXPERIMENTAL METHODS

Cement preparation – Spray dried Hydroxyapatite (HA) powder (SAI, Vaulx-en-velin, FRANCE) was sintered in order to enhance the mechanical properties of the spherical granules (80 µm mean diameter) and at the same time to keep a sufficient porosity to allow remodeling by bone cells. A medical grade α-calcium sulfate hemihydrate (LAFARGE PRESTIA, Meriel, FRANCE) was mixed at different water on powder ratios with a polymeric injection agent. A 10mL syringe was filled by the powders and then the water was aspired to realize the injectable composite. The mix was made by shaking the syringe to obtain an homogenous paste

Porosity test – Small samples of hydroxyapatite powder sintered at different temperature were tested by mercury intrusion porosimetry to characterize their porosity.

Homogeneity test – In order to assess the homogeneity of the injected material, Thermo Gravimetric Analysis was used to characterize the calcium sulfate weight ratio on samples at different levels of injection through a 16 Gauge needle. A 10mL syringe was filed with 4mL of bone cement and a sample was taken after each milliliter injected and dried in an oven at least 16h at 45°C before the test.

Mechanical test – The compressive strength of the cement was measured on cylinders of 15mm in height and 30mm in diameter. These tests have been made after setting of the composite and after drying in an oven at 45°C at least during 16h.

Setting time test – The measurement of the setting time was performed by a Vicat apparatus with a silicon mold (60mm in height and 40mm in diameter). The setting is determined when a 1mm diameter needle loaded by a 300g weight does not leave a mark on the sample anymore.

RESULTS

Porosity test – This test revealed a repartition of the micro-pores interconnection diameter between 1µm and 200nm in the HA sintered at 600°C and 1000°C. Even after a sintering at 1000°C, the HA granules keep a major part of their micro-porosities. A second peak at 20 µm is characteristic of the size of interconnections between the spherical HA granules. After resorption of the calcium sulfate part of the composite, the porosities between the HA granules should be suitable for penetration of bone cells to allow recolonization by new bone (osteoblasts typical size is about 20 µm)[4,5]

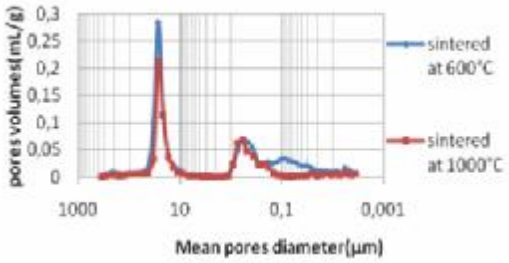

Fig. 1 Pores Volumes and mean pores diameter as a function of sintering temperature

Scanning Electron Microscopy confirmed that the micro-pores in the HA granules were interconnected in the powder after a sintering cycle of 10h at 1000°C and that sintering necks where present between hydroxyapatite particles to allow cohesion of the granules. These interconnections between the porosities are necessaries to

permit the degradation of the granules by the biological fluids.

Homogeneity test – The TGA allowed to dehydrate the calcium sulfate contained in the composite and thus to characterized the weight ratio of CaS in the cement at different levels of injection. The weight loss was the same within every sample tested (cf. fig 1), whatever the percentage of cement contained in the 4 ml syringe injected. This lead to conclude that proportion of CaS is similar from the start to the end of the injection.

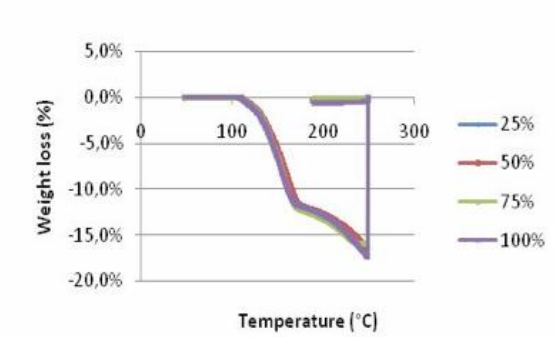

Fig. 2 Weight loss as a function of the percentage of injected cement (on 4 ml)

Working time / Setting Time – A composition made in 50%wt. of hydroxyapatite showed a setting time of 27 minutes. Hardening occurred even if the cement was injected in water. The working time of this composition is more than 6 minutes.

Mechanical Test – The compressive strength of three different compositions were tested. The results are presented in the table below. The higher the hydroxyapatite ratio in the cement, the lower the mechanical properties.

%WT. HAP	MEAN COMPRESSIVE STRENGH (MPA)
50,0%	6,7
45,0%	9,7
40,0%	13,9

Table 1. Compressive strength as a function of %wt. of hydroxyapatite added in the substitute

CONCLUSION

The Mercury Intrusion Porosimetry demonstrated that the porosity between the Hydroxyapatite granules is at least 20 µm and should be suitable for the cell recolonization. Moreover, the micro-porosity of theses granules should be sufficient for the biodegradation by the biological fluids. This mix is easily injectable, homogenous, with a working time and a setting time optimized to fit with the surgery's needs. The compressive strengths of the composite are suitable for the aimed applications. After injection, the calcium sulfate matrix will tend to disappear in a few weeks in the patient's body whereas the hydroxyapatite scaffold will remain. This scaffold should permit the recolonization by bone cells.

ACKNOWLEDGMENTS

This work was supported by MEDICAL BIOMAT, France.

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ACCURACY OF CEMENT DELIVERY WITH A MOTORIZED INJECTION SYSTEM WITH CEMENT CURING

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INTRODUCTION

The delivery of PMMA paste into a host bone has grown significantly for the repair of osteoporotic bones. Appropriate injection technique including placement1, volume2 and viscosity3 of this cement can impact the safety and outcome of the treatment4,5,6, particularly the biomechanical stability of the treated structure1. As such, motorized systems may reduce variability compared to manual techniques. In addition, the novel in-situ monitoring the cement curing, which is based on the cement electric properties, helps physicians detect the beginning of the application phase. The goal of this study was to measure the reliability of the integrated system to power-deliver cement accurately while respecting the limits of the application phase based on the cement's viscosity and improved controls

MATERIALS AND METHODS

The electromechanical cement delivery system was designed to accurately control cement delivery. The system includes real-time monitoring of cement polymerization, injection volume, and more importantly a electromechanical pressure-relief to stop the delivery immediately if needed and thus reducing excess cement overflow. The cement polymerization was measured using cement reservoirs instrumented with the novel dielectric transducer. Injections were performed using Osteopal V cement (Heraeus Medical, Wehrheim, Germany). Displayed volume was compared to measured volume to evaluate accuracy. Cement overflow was evaluated by weighing the cement exiting the delivery needle after halting the injection.

RESULTS

The ability of the novel cement transducer in detecting the optimal viscosity has been validated at room temperatures of 17 to 25oC. The accuracy of delivered cement volume was verified for flow rate of 0.5, 3 and 10mL/min, respectively. The error in the delivery volume was 0.02mL, below 5 percent (SD of 0.05ml). The ability to stop the flow immediately was measured at different degrees of polymerization. The cement overflow following a halt in the injection was 0.085 ± 0.028 mL.

DISCUSSION

The motorized system enables accurate cement delivery with variable delivery speeds. The dielectric system was able to consistently detect the beginning of the working phase of the PMMA cement and thus providing physicians with control on the cement delivery and cement application. In addition, the electromechanical control allows immediate halt of the cement flow if leakage is detected. It is expected that the integration of the above will help the physicians in exerting a more controls of the cement delivery in bone augmentation procedures.

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NOVEL INTEGRATED APPROACHES IN VERTEBROPLASTY WITH UNIPEDICULAR ASPIRATION

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INTRODUCTION

Vertebroplasty techniques made tremendous advances over the last five years. Novel approaches can be used to further improve vertebroplasty safety and procedure1. Cement leak depends on cement viscosity and time of cement delivery has been thus far subjective2-3. The exact amount of injected cements is still not reliable. Also, the physician runs a high risk of radiation exposure, and lastly intravertebral cement distribution may vary and cement may leak1. We present a motorized delivery system that integrates objective polymerization measurements of cement to be injected and a unipedicular bone marrow aspiration technique4. The study verifies in cadaveric sessions the feasibility of this novel vertebroplasty technique designed to provide greater control to physicians and enhanced patient's safety.

MATERIALS AND METHODS

Eight experienced physicians in the vertebroplasty procedure were involved in the feasibility study. The procedure was applied to six cadavers to examine (a) the motorized cement delivery controls, (b) the in-situ cement polymerization monitoring technique, and (c) the aspiration technique for controlled filling and removal of displaced tissue. Under fluoroscopic guidance, needles were placed in the index vertabra in accordance to the current practice. PMMA cement polymerization was monitored through the system and upon reaching the optimal viscosity, the physician controlled the delivery using the remote control unit and thus being outside the radiation field. The injections were performed bilaterally and the injected volume was monitored. Injections were done with and without the unipedicular aspiration technique.

RESULTS

The total of 46 vertebrae were injected without any significant leakage. Physicians injected 2-4mL per pedicle. They expressed a high degree of satisfaction with the motorized controls, and with the ability of the system to repeatedly detect the cement optimal viscosity. The use of aspiration leads to more uniform and predictable filling patterns compared to the control group. In addition, aspiration was able to help to assist physicians in controlling the intravertebral filling and removing displaced bone marrow.

DISCUSSION

The integrated system enhances VP safety, cement distribution and radiation exposure considerably. Those improvements reflect the general change towards vertebroplasty, which is now not only competitive, but largely superior to balloon kyphoplasty in its properties without destroying healthy bone in order to allow cement filling. The integrated system is useful for reducing variability5 in outcomes for less experienced physicians.

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A REVIEW OF MODERATE CONSCIOUS SEDATION AND MONITORED ANESTHESIA CARE IN 477 PATIENTS UNDERGOING PERCUTANEOUS VERTEBROPLASTY

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INTRODUCTION

The aim of this study was to review the use and safety of moderate conscious sedation and Monitored Anesthesia Care (MAC) in patients undergoing vertebroplasty in an interventional radiology setting as we regard this component of the procedure to be a potentially more important cause of morbidity and mortality in an elderly population than the technical causes already published in the literature.

MATERIALS AND METHODS

In the course of our retrospective study, 661 thoracolumbar vertebral compression and sacral insufficiency fractures were treated during 559 vertebroplasty procedures in 477 patients. The patients ages ranged from 45 to 102 yrs (mean 81.1yrs). Three hundred sixty five procedures (65%) were performed on patients 80 years or older. One hundred ninety procedures (33.9%) were performed in patients who were ASA classification 3 or higher

The majority of patients were given moderate conscious sedation in the form of IV Midazolam and Fentanyl in 0.5 mg and 25 mcg aliquots respectively by a trained nurse under the supervision of the radiologist performing the vertebroplasty. Selected patients with ASA 3 status and all patients with ASA 4 status were given Monitored Anesthesia Care, which included Propofol, Ketamine and General Anesthesia, by an Anesthesiologist.

RESULTS

The incidence of periprocedural hypoxia and hypotension was less than 2%. All patients responded to supplemental oxygen and IV fluid respectively. There was no significant difference in the complication rate between the patients given moderate conscious sedation and those who received MAC.

None of the patients required pharmacologic reversal agents, emergency intubation or cardiopulmonary resuscitation. There was no periprocedural mortality.

The 30 day mortality rate was 1.1% due to progression of malignancy in patients who had been treated for metastatic disease to the spine.

CONCLUSION

We believe that with careful patient selection and the appropriate choice and administration of moderate conscious sedation or MAC, vertebroplasty can be safely performed in an elderly population.

FEMOROPLASTY USING AN INJECTABLE AND RESORBABLE CALCIUM PHOSPHATE BISPHOSPHONATE LOADED BONE SUBSTITUTE BY MINI-INVASIVE TECHNIQUE TO PREVENT CONTRA-LATERAL HIP FRACTURE IN THE ELDERLY: A CADAVERIC BIOMECHANICAL STUDY.

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INTRODUCTION

Prevention of hip fracture in the elderly imposes great benefit for care patient as well as for society. The incidence of contra-lateral, second hip fractures after a hip fracture surgery is as high as 20%.
Femoroplasty using an injectable and resorbable calcium phosphate bisphosphonate loaded bone substitute to prevent contra-lateral hip fracture may represent a promising preventive therapy. We aimed to evaluate the biomechanical consequences of the femoroplasty using this new bone substitute.

MATERIALS AND METHODS

Twelve paired human cadaveric femora from donors with a mean age of 86.3 years (7 women and 5 men) were included in this study. One femur from each donor was randomly assigned for femoroplasty and they were biomechanically tested for fracture load against their contra-lateral control. A-P and lateral radiographs and DXA scans were acquired before injection. Femoroplasty was performed under fluoroscopic guidance with an injectable and resorbable bisphosphonate loaded bone substitute. All femurs were fractured by simulating a lateral fall on the greater trochanter by an independent observer. The Wilcoxon's signed rank test was used to test for differences in fracture load between the reinforced femurs and the controls.

RESULTS

Mean T-score of the tested femurs was -3,4 (SD±1,53). All the observed fractures were Kyle II trochanteric fractures. Mean fracture load was 2786 Newton in the femoroplasty group (group F) versus 2116 Newton in the control group (group C) ($p<0.001$). Fracture loads were always higher in the group F : mean 41.6% (mini: 1.2%/maxi:102.1%) and very significant ($p=0.00024$). Effect of femoroplasty was significantly superior for women (+57%) and also correlated to initial BMD ($p<0.0001$). A positive correlation between BMD and fracture load was observed both in control femurs ($R2= 0.74$) and reinforced femurs ($R2= 0.81$).

CONCLUSION

According to our results, femoroplasty with an injectable and resorbable calcium phosphate bisphosphonate loaded bone substitute can provide significant short term biomechanical reinforcement of the proximal femur to prevent osteoporotic contra-lateral fracture.

ACKNOWLEDGMENTS

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X-PRESS BKP: A PRELIMINARY EXPERIENCE

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INTRODUCTION

The aim to study was to evaluate the effectiveness of a new device for balloon kyphoplasty in the treatment of traumatic

vertebral fractures by means of pain reduction and height reastauration.

MATERIAL AND METHODS

From June to October 2009 we performed percutaneous kyphoplasty on 18 patients (5 male, 13 female, average age: 64,4) with traumatic vertebral fractures using the new kit called "xpress" (Kypkon Medtronic). It's characterized by 10 Gauge needles and 10 or 15 mm balloons. For each patients was used a bone cement composed by PMMA (60%) and hydroxyapatite (40%) (Activos, Kyphon Medtronic). All the procedures were executed with local anaesthesia under digital fluoroscopic guidance. A total of 25 vertebras was treated. Patients had been previously evaluated with clinical examination and with X-Ray, CTms and MRI T2w STIR. Clinical evaluation and assessment of pain by means of a Visual Analogue Scale (VAS, 0-10) was performed before the procedure and one month after. Height restauration by means of volume calculation with CTms was made before and after the procedure. It was also rated the degree of patient satisfaction.

RESULTS

We have obtained a progressive reduction of the pain in all the patients (Av. VAS pre: 7.3, av. VAS post 2.9, av. VAS difference: 4.4), a good restoring of the height (Av. h pre: 142mm, av. h post: 157mm. av. h difference: 1.5mm), and a good increasing of the volume of the vertebral bodies (Av. V pre: 15.0cm3, av. V post: 15.7cm3, av. V difference: 0.7cm3). Patients reported a good satisfaction with the procedure. No complications arose.

CONCLUSIONS

From our preliminary studies Xpress BKP resulted to be safe and effective in the treatment of VCF. It also demonstrated to be more feasible: better placement of the needles and shorter procedure with less suffering of the patients is allowed by a thinner working cannula.

EFFICACY OF PERCUTANEOUS VERTEBROPLASTY WITH CALCIUM SOLFATE: A PRELIMINARY EXPERIENCE

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INTRODUCTION

The aim to study was to prove the effectiveness of a bone subsitute (Cerament, Bone Support, Sweden) in the treatment of osteoporotic or traumatic vertebral fractures with percutaneous vertebroplasty.

MATERIAL AND METHODS

From March to October 2009 28 patients (10male, 18female, average age:53,2) underwent percutaneous vertebroplasty. We treated 57 vertebral bodies by means of injection of Cerament (calcium solfate60%+hydroxyapatite40%) through 10 and 13Gauge bevel-edge needles (Gangi type). About 3cc of bone cement was injected for each vertebra. Patients had been previously evaluated with clinical examination and with X-Ray, CTms and MRI T2w STIR. All the procedures were executed with local anaesthesia under digital fluoroscopic guidance.
Clinical evaluation and assessment of pain by means of a Visual Analogue Scale (VAS, 0-10) and a Oswestry Disability Index (ODI, 0-50) test was performed before and one month after the procedure. We also carried out X-Ray, CTms and MRI checks at one, three and six months.

RESULTS

We have obtained a progressive reduction of the pain in all the patients (Av. VAS pre: 7.83, av. VAS post 1.66, av. VAS difference: 6.17) and a good improvment of the quality of life (Av. ODI pre: 22.81. av. ODI post: 8.64, av: ODI difference:

14.17), a progressive reduction of bone edema evaluated through MRI T2w STIR and a progressive inclusion of the bone cement evaluated through CTms. No complications arose.

CONCLUSIONS

Our preliminary studies prove that calcium solfate could result in being an actual substitute of PMMA in the treatment of osteoporotic and traumatic vertebral fractures, especially in young patients.

VERTEBROPLASTY - RATIONALE (AUT IRRATIONALE?)

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INTRODUCTION

Purpose of our work is to use the elementary knowledge about rational mechanic and biomechanic to create solutions about trouble concerning percutaneous treatment of vertebral fractures.

MATERIALS AND METHODS

Retrospective analysis of 2 cases of vertebral osteonecrosis (Kummel's Disease) treated by percutaneous antalgic vertebroplasty.

RESULTS

Using our knowledge of years of percutanoeus antalgic vertebroplasty and especially what it concerns about this procedure in two cases of vertebral osteonecrosis (Kummel's Disease), we confirm what is described in biomechanical studies of many authors since 1700. We know very well how nature can reach perfection, using minimal resources.

Rational finalistic optimization of biological structures, in particular loading weight, either still or dynamic helped us to evaluate the effectiveness of the distribution of materials injected in malacotic and neoplastic vertebras. When is it time to stop the injection and how we have to stop injecting? And how much material is correct to solve the pain?

In Our two specific cases, we got antalgic effects in the same way for both, even if cement allocated itself complementary. (Case 1: cement only in necrotic area; Case 2: cement only around necrotic area).

Our study should balance distribution of forces and materials, anyway happening in nature due to either still or dynamic demands and floodings.

Re-estabilishing a biomechanical equilibrium in vertebras is not the same as re-build it; First favoring natural re-distribution of loads in vertebral fractures is essential, without contrasting physiological evolution.

CONCLUSION

Many questions to medical and bioengineering Research are obvious: Which materials could collocate themselves following force lines better?

Are we able to determine a quantity limit of usable cement or resin?

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POSTOPERATIVE TREATMENT COSTS AFTER CEMENTOPLASTY, A COMPARATIVE ANALYSIS TO CONSERVATIVE TREATMENT AFTER OSTEOPOROTIC VERTEBRAL FRACTURES

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INTRODUCTION

The development of injectable biomaterials is based on the assumption that they will also be applied in surgery. However, those biomaterials in general increase perioperative costs, which are still covered under the DRG codes. Nevertheless new and more expensive biomaterials need more arguments and discussions with clinical administrators.This paper may help in those discussions comparing the postoperative costs after cementoplasty to a, assumedly cheaper, conservative treatment.

MATERIALS AND METHODS

110 patients after conservative treatment and 141 patients after balloon kyphoplasty treated primarily between 2002 and 2005 in one center were followed up via a nationwide analysis of spine related inhospital treatment.

Data from the Austrian DRG-system, which includes all inpatients treated in Austria have been used to identify admission of the target population between 2002 and 2006. number of admissions, the length of stay and the scores can be determined. Furthermore each admission was classified as spine related or not.

The data were matched against the Austrian death registry. If a patient has died this data was used to calculate the follow up time otherwise December 31st 2006 was used.

The mean age of the conservative group was 75.49 and of the kyphoplasty group 71.16 years.

The total follow up time was 324.55 years(mean +-standard deviation 2.92+-1.40) for the conservative and 354.25 (2.53+-0.96) for the kyphoplasty group.

RESULTS

The mean number of admissions in the kyphoplasty group is 0.779 or 0.308 per follow up year whereas in the conservative group these figures are approximately twice, namely 1.757 and 0.601. Considering the average length of stay the kyphoplasty group shows less inhospital days (9.2 per patient or 3.6 per follow up year), whereas in the conservative group this is 14.4 and 4.6 inpatient days. Finally the scores per admission in the follow up period are lower in the kyphoplasty group (3146 and 1243 DRG related treatment points) whereas in the conservative group these values are 3824 and 1308.

CONCLUSION

These data show a strong superiority of kyphoplasty compared to conservative treatment of spine problems based on the data of one big hospital in Austria where 251 patients have been treated between 2002 and 2005.

We demonstrate a long term superiority of balloon kyphoplasty compared to non-surgical treatment regarding inpatient treatment which is an indication that cementoplasty is saving costs in a long term and the development of future, less toxic materials assuring lower perioperative morbidity is worthwhile.

ACKNOWLEDGEMENT

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KYPHX HV-R, ACTIVOS TM AND KYPHOS TM IN VERTEBROPLATIC AND KYPHOPLASTIC PROCEDURES

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INTRODUCTION

This work was made specially for the Griboi meeting. The aim is to show the different characteristics and the use of these tree material in vertebral bodies fracture, by trauma, osteoporosis and osteolitic lesions.

MATERIALS AND METHODS

We will not keep any secret. In our department I had treated 850 pt in 5 years with different materials with vertebro-plastic and kyphoplastic procedures. 90 pt treated with these tree materials were enrolled: 30 pt. treated with Kyph HV-R, 54 lumbar, 33 dorsal vertebral bodies; 30 pt with ActivOS, 46 lumbar, 16 dorsal levels; and 30 pt with KyphOs FS, 55 lumbar and 19 dorsal levels. The pain was measured with VAS and ODI score, pre and post procedures. Pre operatory exams were RX plain film of dorsal and lumbar column, CT or MR of vertebral body lesions. All Patients were treated in day-surgery in local anesthesia with radiological view using flat panel. After procedure patients underwent a clinical visit immediately post surgery procedure; after 10-15 days, clinical visit and RX of dorsal and lumbar spine at 1,3,6,mth and at 1 year.

RESULTS

We'll show chemical and physical characteristics of the materials, the key points procedure.

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CERAMENT: OSTEOCONDUCTIVE MATERIAL: USE IN VERTEBROPLASTIC AND KYPHOPLASTIC PROCEDURES

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INTRODUCTION

This work was made especially for the Griboi meeting. The aim is to show the chemical and physic characteristics of this osteo conductive material to make a new bone in osteoporosis and fractures of vertebral bodies. After the injection of the material is possible to obtain a new bone in 18-24 months.

MATERIALS AND METHODS

We will not keep any secrets. There were studied 40 pt, with osteoporotic or trauma vertebral bodies fractures, in sub acute phase with pain and edema in the vertebral body fractures. 20 pt of them underwent a vertebroplastic procedure and 20 underwent a kyphoplastic procedure. Pre operatory exams were the following: clinical visit, using VAS and ODI score, RX plain film, CT and MR. In the follow-up were done the same exams to evaluate the clinical results.

RESULTS

The all procedures were well done without no main or minor complicances, the VAS and ODI score decreases in all cases. This new material can be used in sub acute and chronic vertebral body fracture with indication to treat; we will show the characteristics, the key point and the results. It cannot be use in acute vertebral fracture phase and fracture with secondary lesions (mets, Myeloma, Leukemia).

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PERCUTANEOUS ANTERIOR COLUMN STABILIZATION OF

FOCAL METASTATIC LESIONS OF THE SPINE: THE VALUE OF PLASMA-MEDIATED RADIOFREQUENCY ABLATION BEFORE CEMENT INJECTION

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INTORDUCTION

Spinal metastatic lesions which are located in the anterior or posterior regions of the vertebral body (Weinstein Types III and IV) are usually treated by extensive anterior corpectomy stabilization and posterior fusion. This study assesses the value of creating a cavity in the anterior 2/3 of the vertebral body using plasma-mediated radiofrequency ablation prior to cement injection, aiming to stabilize the anterior column through a non-heat-driven method.

MATERIALS AND METHODS

Retrospective assessments of CT images were performed pre- and post-procedure in 24 patients (27 levels). A void was created in the anterior portion of the tumor-infiltrated vertebral body using a bipolar plasma-mediated radiofrequency-based wand (ArthroCare Corporation, Austin, TX) and bone cement was inserted. Pain was recorded both pre-procedure and 2-4 weeks afterwards using a visual analogue scale (VAS).

Results

In 11/12 levels with anterior lesions, cement was deposited inside the lesion. Cement was also successfully deposited anterior to the lesion in 13/15 levels with posteriorly-located lesions. VAS pain scores were available for 21 patients: 19 reported significant pain relief while 2 exhibited no change. No clinically significant leakage was observed.

CONCLUSION

Cavity creation using plasma-mediated radiofrequency ablation can be performed percutaneously before standard vertebroplasty, and results in more control over cement deposition in the anterior part of the vertebral body, regardless of the lesion location. This approach could treat focal metastatic lesions while avoiding the more invasive standard technique of extensive anterior surgical debulking and reconstruction. In cases displaying neurological deficit, it can complement a simpler posterior decompressive laminectomy and fusion.

PERCUTANEOUS VERTEBROPLASTY FOR OSTEOPOROTIC FRACTURES: EXPERIENCE WITH HIGH VISCOSITY CEMENT USING A HYDROLIC INJECTION DEVICE, THE "CONFIDENCE" SYSTEM.

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INTRODUCTION

Vertebroplasty is a widely used technique to treat painful osteoporotic vertebral compression fractures, however, precise control of cement delivery is necessary to minimize the risk of cement leakage. The study is conducted to assess the clinical feasibility of performing vertebroplasty on osteoportic compression fractures using an ultraviscous cement injected by a hydrolic device, CONFIDENCE Vertebral Augmentation System, to further control cement deposition.

MATERIALS AND METHODS

A retrospective evaluation of series of 94 consecutively treated patients were identified for the review. There were a total of 163 levels which ranged from T3 to L1 vertebral bodies. The degree of leakage, seen in the post-operative films, was assessed at each treated level using a strict 4-point scale (none, minimal, moderate, severe). The pattern of any observed leakage was also characterized

as: discal, venous, paravetebral, or epidural.

RESULTS
Pre-operatively the mean degree of vertebral collapse was 29%. A bi-pedicular approach was used for 82% (133/163) levels and unipedicular in 18% (30/163). There was no leakage in 50%, minimal leakage in 42%, and moderate leakage noted in 8% of cases. Both unipedicular and bi-pedicular approaches showed leaks in 50% of cases. The most frequent pattern of leak was venous indicated in 52% of leaks, the adjacent disc in 46%, and paravertebral in 5%. The pattern of leakage was always limited to one region except in two cases.. There were no symptomatic leaks that required surgical intervention.

CONCLUSIONS
Vertebroplasty in osteoporotic fractures using a highly viscus cement that can be safely controled and injected via an hydrolic system can be performed safely without significant complications

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PERCUTANEOUS CEMENT AUGMENATION FOR TREATMENT OF METASTAIC LESIONS OF THE SPINE AND PELVIS USING A NOVEL HIGHLY VISCOUS CEMENT: EXPERIENCE IN 17 CONSECUTIVE PATIENTS

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INTRODUCTION
Percutaneous cement augmentation had been described for treatement of painful metastaic lesions of the spine and pelvic bones using standard Vertebroplasty systems usually with low viscosity cements have been associted with a higher percentage of leaks compared to benign fractures. The study was done to assess the safety, feasibility, and clinical outcome of using a higly viscous cement injected using a hydrolic based device in treatment of metastaic lesions of the spine and pelvis using flouroscopy and Computed Tomography guidance.

MATERIALS AND METHODS
A series of 17 consecutively treated patients with different types of different primamry and secondry tumors were identified for the review. There were a total of 29 lesions treated. Levels treated ranged from C7 to L5, sacrum, ilium, and ischium.The degree of leakage was assessed at each treated level using a strict 4-point scale (none, minimal, moderate, severe). The pattern of any observed leakage was also characterized as: discal, venous, paravetebral, epidural, or SI joint. Cement leaks were assesed using both plain films and CT. Visual analog score (VAS) for pain was collected before the procedure and within 2-4 weeks after.

RESULTS
On plain film there was no leak at 13, minimal at 9, and moderate at 2 levels. On CT there was no leak in 4, minimal at 15, and moderate at 2 levels. CT imaging identified 10 additional minimal leaks, but did not identify any additional moderate or severe leaks.
VAS pain data was available pre and post-procedure for 10 of 17 patients. The mean VAS pain score improved 49% from mean pre-op of 8.2 to a mean post-op VAS was 4.2.

CONCLUSIONS
Percutaneous cement augmentation of metastic lesions of the spine and pelvis is feasible and safe using a high viscosity cement system. A hydrolic injection device allow more control over the injected cement into a small caliber needles. Cement augmentation results in pain relief as measured by VAS with a 49% decrease in pain score.

VERTEBROPLASTY WITH CONTROLLED CEMENT VISCOSITY REDUCES LEAKAGE

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INTRODUCTION

Vertebral compression fractures are routinely treated with vertebroplasty. The first source of complications during this procedure is cement leakage either into the extraosseous space eventually causing neurological deficits or even pulmonary embolisms. Leakage rates have been reported frequently, with a wide range (40-89%) depending on the indication (osteoporotic fracture, osteolysis in tumor diseases), number of levels treated and the technique used to quantify leakage. The most important parameter that influences leakage is cement viscosity [1]. Therefore, appropriate timing of cement injection is of ultimate importance as the viscosity increases with time. An early injection with low viscosity has a high risk of leakage, while late injection with high viscosity reduces time of injection and hinders appropriate cement delivery into the vertebra.

MATERIALS AND METHODS

The VISCOSAFE Viscometer, which is part of the Vertecem Vertebroplasty System, was used to control viscosity of the cement. From August 2007 to October 2009, 60 patients (36 females and 24 males) suffering from osteoporotic fractures, myeloma or metastatic lesions were augmented with Vertecem. Average patient's age at time of surgery was 68 ± 11 years. A total of 92 levels (T6 - S1) were augmented with on average 3.87 ± 0.84 ml cement.

RESULTS

Leakage was assessed by X-rays and CT scan, and observed in only 12 out of 92 treated levels (13%), respectively. In six patients, the cement penetrated into the extraosseous space of the vertebral disc, while in five subjects the cortical veins were affected. One treatment resulted in leakage into both disc space and epidural vein. Neurological status were not compromised.

CONCLUSION

The Viscometer of the Vertecem Vertebroplasty System is a reliable tool to determine the ideal time for cement injection, resulting in a low leakage rate. Leakage rates are decreased, thus increasing the safety of the procedure. Side-opening vertebroplasty needles is another benefit of the system since the cement flow can be directed as desired. The Vertecem System shows satisfactory clinical performance and meets the surgeons' demands.

REFERENCE

Baroud G., Crookshank M., and Bohner M. (2006). High-viscosity cement significantly enhances uniformity of cement filling in vertebroplasty: an experimental model and study on cement leakage. Spine (Phila Pa 1976.) 31: 2562-2568.

SUITABILITY OF A CALCIUM ALUMINATE CERAMIC IN BALLOON-KYPHOPLASTY OF OSTEOPOROTIC VERTEBRAL BODY FRACTURES. RESULTS OF A PROSPECTIVE CLINICAL TRIAL

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INTRODUCTION

In kyphoplasty and vertebroplasty, polymethyl methacrylate (PMMA) is currently the standard in augmentation

materials but it is characterized by a lack of osseointegration and limited biocompatibility.
At the same time, calcium phosphate cements are not currently considered an alternative due to their insufficient mechanical stability against shear, compression and extension forces.
This prospective study examines the suitability of a new calcium alumiate ceramic (Xeraspine(r), Doxa) for augmentation of vertebral fractures treated with balloon-kyphoplasty.

MATERIALS AND METHODS

Surgical technique follows the standard protocol for bilateral percutaneous balloon-kyphoplasty (Medtronic). Exclusion criteria were: additional instrumentation, age > 90 years, as well as cardiac arrhythmia. The following clinical and radiological data were collected pre and post op, after 6 weeks, and after 3, 6, 12 and 24 months: Visual-Analogue-Score, Oswestry-Disability-Index, bisegmental endplate angle, and vertebral hight. CT-scans were provided to show possible cement extrusion and disintegration.

RESULTS

26 patients with 30 fractures were included. The fractures could be classified as type A1.3 (n=15) and A3.1 (15). They were located at Th9 - L3. All 26 patients reported pain relief immediately post op. Restoration of endplate angles was 5,8° on average. There were three cases of cement disintegration occuring after 6 month with radiological loss of correction. All three cases were fracture types A3.

CONCLUSION

The calcium aluminate ceramic used in balloon-kyphoplasty is an alternative to PMMA in less-challenging osteoporotic fracture-types A1.3. There is , however, a risk of cement failure and following loss of correction in fracture types A3. Because of this, the cement tested is only recommended for fracture-types A1.3.