

Development of a Resorbable Calcium Phosphate Cement with Load Bearing Capacity

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Abstract

Compared to cortical bone and polymeric bone cements, the mechanical properties of calcium phosphate cements are generally poor. This has resulted in them being used in non-load bearing clinical applications. The aim of this study was to investigate the possibility of producing a brushite cement with mechanical properties closer to those of cortical bone (i.e., >100 MPa in compression), i.e. with a potential to be used in load bearing applications. With a compressive strength of 74.4 (\pm 10.7) MPa, maximum at 91.8 MPa, the cement presented herein is comparable with the non degradable polymeric counterparts and the strongest hydroxyapatite cements, and is close in strength of cortical bone. Furthermore, it has a high injectability (>90%) and a setting time of approximately 17 minutes. A cement comprising these properties has great potential of changing the future clinical indications for calcium phosphate cements, and could potentially reduce the use of non-degradable polymeric cements.

Keywords: Brushite; Cement; Calcium phosphate; Strongest

Introduction

Due to a rapidly aging population, the use of implants is increasing. An increasing number of implants are also expected to remain and function within the host for longer periods of time. The risk of complication due to implant failure increase with implantation time, and thus resorbable material options (where appropriate) represent an important challenge. Materials that are injectable, can carry a load, slowly resorb over time, and allow for bone in-growth represent a significant development compared to the current biomaterial options. Both polymers and ceramics have been evaluated for this purpose. It is however difficult to find suitable polymer chemistry that also fulfills demands on biocompatibility and degradability. Calcium phosphate cements (CPCs), which are highly biocompatible, represent a good starting point, but are limited by their low strength and ductility. At present the interest in CPCs is high, and there are many products available on the market [1]. However, the main use of CPCs is as non-load bearing bone void fillers where the experienced stresses are limited (e.g., craniofacial applications [2,3]) or where they can be used together with external fixation (e.g., orthopedic applications [4]). Depending on the pH during CPC setting reaction (acidic, or neutral to basic), two main phases are found as the precipitated end product; brushite (acidic) or hydroxyapatite (HA, neutral to basic). Optimization of the strength of CPCs have been performed by a few groups, with results in compression of around 80-100 MPa for HA cements [5,6], and about 50 MPa for brushite cements [7]. It has furthermore been shown that the strength can be even higher if a load is applied during molding of the paste [6]. The results achieved for HA cements is in the vicinity of the strength of cortical bone, which reaches around 100 MPa or higher in compression [8]. Although HA is similar to the mineral phase of bone (ion substituted calcium deficient hydroxyapatite), brushite is a metastable phase with a higher solubility at neutral pH [9]. Brushite cements have also been shown to have a high biodegradability, both in vitro and in vivo [10,11] compared to HA cements, which show little or no resorption over long periods of time [10].

The starting materials for brushite cements is normally beta-tri-calcium phosphate (β -TCP) mixed either with monocalcium phosphate monohydrate (MCPM) [12] or phosphoric acid [13]. The starting powders are all stable at room temperature, and are therefore

cheaper to produce than the metastable phases used as starting powders for HA cements.

Improving the strength of a ceramic material, a cement in particular, can be made by different routes; e.g., decreasing the porosity, decreasing the crystal size, adding a filler material with good mechanical properties, and optimizing particle sizes of the precursor powders. Reduced porosity can be achieved in a few ways; by decreasing the liquid-to-powder ratio (L/P) [5,7,14], by reducing the amount of air incorporated into the cement during mixing, or by compaction of the paste during molding [6]. Increased strength by decreasing the particle size [13,15-18] can be achieved by incorporation of additives, such as citric acid or different pyrophosphates, which reduce the rate of grain growth and promote the formation of many small crystals [19]. Optimization of the particle size of the precursor powders has been seen to highly affect the mechanical strength of CPCs, both the HA [9] and brushite [7] cements. It could be due to better compaction and lower porosity when optimal ratios are used, and to the speed of dissolution that optimally should be the same for all components, giving that the powder with lower solubility should have smaller particles than the component with the higher solubility [9]. From the correlation between strength and porosity it has been hypothesized by Hofmann et al. [7] that the maximum achievable strength in compression for brushite cements is around 83 MPa.

In this study we present compressive strengths above the previously reported values for the degradable brushite cements. For clinically relevant brushite cement with highest strength possible,

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minimizing the L/P was deemed more feasible than employing external compaction. Furthermore, we chose to use a Cap-Vibrator for the mixing, and used what we believe is a good particle size ratio between β -TCP and MCPM, to achieve a good powder compaction together with an optimal dissolution speed. The MCPM to β -TCP ratio and the additives used were chosen with regard to previous publication [14]. The cement presented herein has been thoroughly analyzed with regard to mechanical and physiological properties.

Materials and Methods

Cement preparation

MCPM (Scharlau, CA0211005P, batch 12371601, Spain) sieved to $<75 \mu\text{m}$, and β -TCP (Sigma-Aldrich, 21218, batch no. BCBH 6869V, Germany, containing around 8-10 wt% beta-calcium pyrophosphate (β -CPP)), as received, were mixed in a 45:55 molar ratio together with 1 wt% disodium dihydrogen pyrophosphate (SPP, Sigma-Aldrich, 71501, batch no. 1103557, Germany). Citric acid (0.5 M (aq)) was used as the liquid phase in an L/P of 0.22 ml/g. In order to reduce the porosity by reducing the amount of air trapped inside the paste during mixing, the mixing was performed twice for thirty seconds in 50 mL falcon tubes, using a Cap-Vibrator (Ivoclar Vivadent, Liechtenstein).

The cement paste was transferred to rubber molds and was allowed to set for five minutes in room temperature ($22^\circ\text{C} \pm 1^\circ\text{C}$) before being immersed in phosphate buffered saline (PBS, Sigma-Aldrich, 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4) or water in sealed plastic containers. The containers were stored at 37°C for 24 hours after which the samples were removed from the molds and prepared for respective analysis.

Mechanical testing

Rubber molds with \varnothing 6 mm and height 13 mm were used to prepare samples for compressive strength (CS) measurements. After storage in 40 ml of PBS the samples were polished using 800 grit SiC paper to make the sides flat and parallel and achieve a height of 12 mm according to ASTM F451 standard [20]. Diametral tensile strength (DTS) samples were made in rubber moulds of \varnothing 8 mm and height 3 mm, with six samples cured together in 80 ml of PBS. Wet CS and DTS were measured using a universal testing machine (Shimadzu, AGS-X, Japan), with a cross-head speed of 1 mm/min. A thin plastic film was placed between the sample and the cross-head in order to reduce the effect of surface defects from molding and polishing.

Setting time

The cement paste was molded in Teflon® rings, with \varnothing 12.5 mm and height 5 mm opened at both ends, and were immersed in 80 ml of 37°C PBS five minutes after mixing. The surface of the cement was tested every three minutes, and the cement was considered to have set when a visible mark could not be seen on the sample after a 453.5 g Gillmore needle with a tip diameter of 1.06 mm (equivalent to a stress of 5 MPa) had been placed on the surface, according to ASTM C266 – 99 standard [21].

Porosity

Volume of the samples (approximately \varnothing 6 mm and height 13 mm) was measured using Archimedes principle on wet samples. Drying was performed in vacuum at room temperature ($22 \pm 1^\circ\text{C}$) for 24 h. The dry samples were weighed and the apparent density was calculated. The skeletal density was measured using helium pycnometry (AccuPyc 1340, Micromeritics, UK), 20 purges and 10 runs. The porosity was

calculated according to equation 1, where Φ is the porosity in percent, ρ_a is the apparent density, and ρ_s is the skeletal density.

$$\Phi(\%) = \left(1 - \frac{\rho_a}{\rho_s}\right) \cdot 100 \quad (1)$$

Microstructure

The microstructure of fractured surfaces was studied using scanning electron microscopy (SEM, LEO 1550, Zeiss, Germany). The samples were sputtered with a thin gold/palladium coating to avoid charging during imaging.

pH

Samples with \varnothing 6 mm and height 13 mm were stored in either 40 ml of PBS or 40 ml of water at 37°C for 24 h, after which the pH of the solutions were measured using a pH/ion meter (S220 SevenCompact™, Mettler Toledo, Switzerland).

X-ray diffraction

Samples from the porosity measurements were thoroughly ground. The XRD analysis was performed using a D8 diffractometer (Bruker, USA) in a theta-theta setup with Cu-K_α irradiation. Diffraction angles (2θ) 5 - 60° were analyzed in steps of 0.02° with 1 second per step and a rotation speed of 60 rpm. Rietveld refinement with BGMN software (BGMN, Germany) was used to calculate the phase composition, with the reported result being the mean of three measurements with the relative error as $2.77 \times$ standard deviation according to ASTM E177 – 13 [22]. The phases used for the refinement were; monetite from PDF #04-009-3755 [23], brushite from PDF # 04-013-3344 [24], β -TCP from PDF # 04-008-8714 [25], MCPM from PDF # 04-011-3010 [26] and β -calcium pyrophosphate (β -CPP) from PDF # 04-009-3876 [27].

Injectability

The injectability was calculated using a method previously described by Gbureck et al. [5]. The paste was transferred to a 3 mL syringe with a barrel diameter of 8.55 mm and an outlet diameter of 1.90 mm. The extrusion was made three minutes after the start of mixing and a universal testing machine (Shimadzu AGS-X, Japan) at a crosshead speed of 100 mm/min was used for the extrusion. A maximum force of 100 N was allowed. The amount of cement extruded from the syringe was weighed and the injectability (I) in percent was calculated according to equation 2.

$$I(\%) = \frac{\text{Mass of paste extruded from the syringe}}{\text{Initial mass of paste in the syringe}} \cdot 100 \quad (2)$$

Results

The CS reached $74.4 (\pm 10.7)$ MPa and the DTS $10.2 (\pm 0.8)$ MPa,

Analysis	Result	Max	No of samples
CS (MPa)	74.4 (10.7)	91.8	14
Youngs modulus (GPa)	2.8 (0.2)		
DTS (MPa)	10.2 (0.8)	11.9	13
Porosity (%)	13.4 (0.7)		8
Setting time (min)	16.8 (1.7)		6
pH (24 h in water)	4.28 (0.09)		4
pH (24 h in PBS)	6.54 (0.07)		8
Injectability (wt%)	92.2 (0.9)		3

Table 1: Results from the material characterization; standard deviations are indicated within brackets.

(Table 1). The corresponding porosity was about 13%. The highest measured strength in compression was, however, as high as 91.8 MPa. Young's modulus measured in compressive loads was slightly below 3 GPa. The microstructure analysis show that there were many small pores (approximately 25-50 μm in diameter) distributed evenly over the sample surface (Figure 1). The grains were flake like, ranging between 100 nm – 1 μm in diameter and approximately 40 nm in thickness. The cement showed a high injectability of >90 wt%, and no obvious filter pressing during ejection was noted. The final setting time of the cement was about 17 minutes, and the cement showed close to neutral pH levels after 24 hours of storage in PBS. However, when stored in water, the pH was around 4 after 24 hours of storage. The XRD analysis of dried samples showed that there still was a total of about 17 wt% un reacted β -TCP and β -CPP in the cement, while all MCPM had reacted (Table 2 and Figure 2). The main precipitated product was brushite, with around 5 wt% of monetite present.

Discussion

The cement presented herein show surprisingly high mechanical strengths, with maximum CS of around 92 MPa, which is almost 10 MPa higher than the reported theoretical maximum value for brushite cements with zero porosity [7], and to the authors knowledge, almost twice as high as previously published values for brushite cements. The

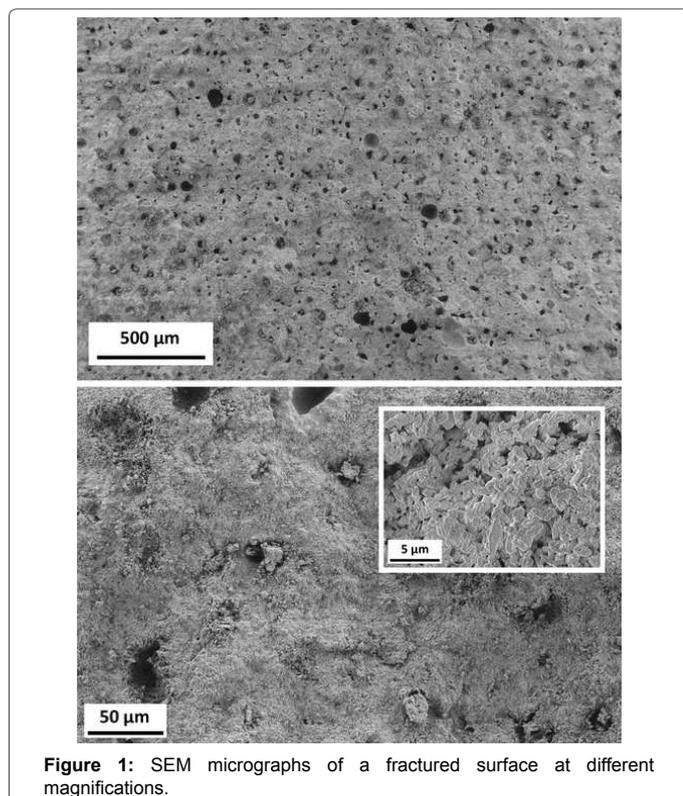


Figure 1: SEM micrographs of a fractured surface at different magnifications.

Phase	Composition (wt.%)
β -CPP	6 (1)
β -TCP	11 (<1)
Brushite	78 (1)
MCPM	0 (<1)
Monetite	5 (<1)

Table 2: Phase composition from XRD and Rietveld refinement. Four samples were analyzed; relative errors are indicated within brackets.

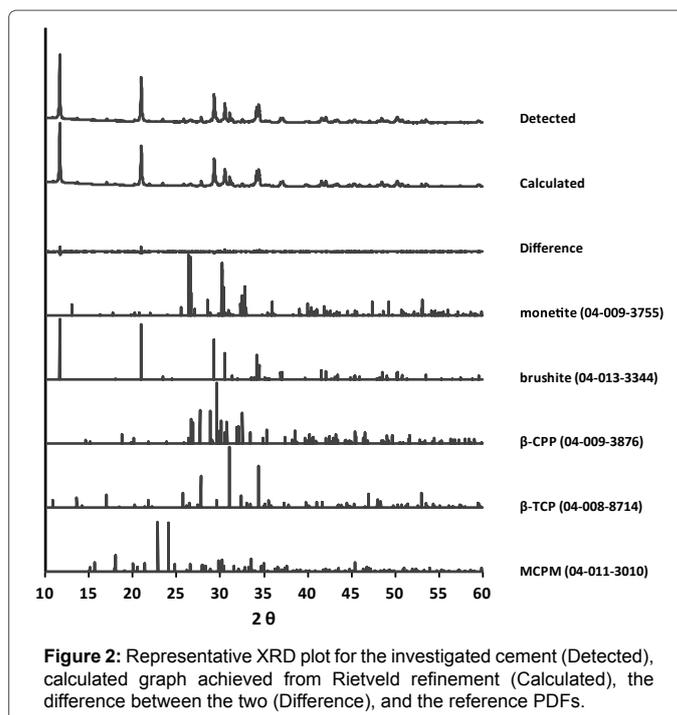


Figure 2: Representative XRD plot for the investigated cement (Detected), calculated graph achieved from Rietveld refinement (Calculated), the difference between the two (Difference), and the reference PDFs.

measured CS is, furthermore, close to the values for CS of cortical bone [8], and in the same range as the strength of polymeric bone cements (ranging between 70-120 MPa [28]). The high value achieved is likely due to a good ratio between cement paste and unreacted β -TCP, which induces a good compaction during the cement reaction, together with the toughening effect of small and hard unreacted β -TCP particles. The Young's modulus measured during compression was just below 3 GPa, which also is similar to the values of polymeric bone cements [28]. This value is lower than the values of cortical and trabecular bone, ranging between 10-25 GPa, with trabecular bone having lower values than cortical [29-31]. The DTS measured on these cements are similar to DTS values reported for HA cements [32,33]; however, being around one tenth of cortical bone [34,35]. One of the main advantages with CPCs is their inherent injectability, meaning that they can be introduced to the site of the defect without invasive surgery. A CPC with high injectability is thus a requirement. Due to the hardening nature of the CPC, the injectability differs with how soon after start of mixing the injection is performed, and how fast the injection is performed. The cement in the present study was almost completely injectable when the injection started three minutes after the start of mixing and took less than one minute to perform. Surprisingly, the setting of the cement did not affect the pH of the PBS drastically, which would be expected from cements utilizing the acidic MCPM together with citric acid. The low effect on pH is likely due to the excess basic β -TCP, stabilizing the pH.

Conclusions

In this paper we have demonstrated an improved brushite cement formulation, with unexpectedly high mechanical strength. The compressive strength achieved after setting was on average 74.4 (\pm 10.7) MPa, with a maximum of 91.8 MPa, which is just below the strength of cortical bone, and to be best of authors knowledge, the highest strength published. Other important properties such as setting time and injectability showed results well within the desired spans, resulting in cement with great mechanical and physical properties. These superior mechanical properties imply that the investigated cement has a great

potential to be used in selected load bearing clinical applications.

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