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A SELF-SETTING, MONETITE ($CaHPO_4$) CEMENT FOR SKELETAL REPAIR

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ABSTRACT

A low compressive strength (2 to 4 MPa) but very simple and inexpensive orthopedic $CaHPO_4$ cement was developed by using commercially available $Ca(OH)_2$ powders as the only starting powder component. The setting solution used was a special aqueous phosphoric acid (H_3PO_4) solution, with a small amount of sodium hydrogen carbonate ($NaHCO_3$) dissolved in it. Calcium was provided by the powder component, and all of the phosphate came from the setting solution. The setting solution was acidic, and this helped to neutralize $Ca(OH)_2$ only to the extent of forming the phase of dicalcium phosphate anhydrous (DCPA, monetite), $CaHPO_4$. At an L/P (liquid-to-powder) ratio of 1.54 and Ca/P molar ratio of 0.9, the cements had an initial setting time of 19±2 minutes. Set cements comprised only crystalline $CaHPO_4$. Monetite has a higher solubility than octacalcium phosphate, β-tricalcium phosphate and calcium hydroxyapatite in aqueous solutions at the physiological pH. This new cement was considered to exhibit a higher *in vivo* resorbability in comparison to the apatitic cements. Cement samples were characterized by XRD, FTIR and SEM. Compressive strength, initial and final setting times (with the use of Gillmore needles) of the cement samples were also reported.

INTRODUCTION

Beyond the autologous bone grafts, synthetic calcium phosphates (CaP) are the materials of choice for skeletal repair, mainly due to their biocompatibility, bioactivity, osteoconductivity, and even bioresorbability (i.e., osteoclast cell-mediated resorption). CaP based cements were first commercialized about two decades ago[1,2]. Self-setting or self-hardening (upon reacting the supplied CaP-based powder component with a special setting or starter solution) CaP cements provided the orthopedic surgeon with a valuable tool of filling three dimensional bone defect within minutes in the surgical theater. Until that time preshaped hard blocks or pieces of CaP were needed to be chiseled and hammered down by the surgeon to the suitable shape and size in accordance with the defect peculiarity.

Most CaP-based orthopedic cements available today were designed to set into calcium hydroxyapatite [HA, $Ca_{10}(PO_4)_6(OH)_2$] as their end products, mainly because of the so-called similarity of HA with the mineral portion of human bones. Hydroxyapatite has the lowest solubility among all the calcium phosphate phases.[1] Moreover, as a result of recent *in vivo* tests,[3,4] such cements based on HA were shown to suffer from low *in vivo* resorbability, and lacking full participation in the bone remodeling processes even a year after their implantation. HA-based cements typically used α-tricalcium phosphate [α-TCP, α-$Ca_3(PO_4)_2$] or tetracalcium phosphate [TTCP, $Ca_4(PO_4)_2O$] as their major constituents. These phases both are able to undergo hydrolysis, into apatitic calcium phosphates, when brought into contact with aqueous solutions. Nevertheless, α-TCP (or TTCP) powders cannot be synthesized at room temperature, and both are fully stable only at high temperatures (in excess of 1200°C), therefore, these high-

temperature phases must be quenched to the ambient temperature, by using quite capital-intensive manufacturing investments.[5-7] The involvement of high temperatures in synthesizing α-TCP and TTCP powders also places a burden on the particle size of these important cement ingredients. Typically, quenched chunks or hard agglomerates of those materials require a delicate grinding operation, which may be a further source of contamination, or premature and undesired interaction of the powders with humidity (to undergo through a smaller extent of particle surface-limited hydrolysis) present during grinding. As a result, the reactivity of these powders would also be a major point of concern[8].

Currently, CaP cements are only available in two categories; (i) apatitic[1,2,9] and (ii) brushitic[10-12] [dicalcium phosphate dihydrate, DCPD, $CaHPO_4 \cdot 2H_2O$], based on the final phase of the set cements. Recent interest in the development of CaP cements is shifting more towards brushitic cements due to their proven high bioresorbability[13]. The bioresorption rate of brushitic cements, i.e., their capability to take part in the *in vivo* bone remodeling processes, was found to be significantly higher than that of apatitic cements[14].

However, all of the currently known cement formulations were based on a powder component which consists of an intricate mixture of at least two or more CaP or Ca- or P-containing phases[15], usually some of those ingredients are basic and the remaining are acidic in nature. Upon achieving the first contact with the setting solution, neutralization reactions start to take place in the powder components to form the apatite- or brushite-like end phases. Such a mixture of CaP may undergo a solid state reaction, limiting the shelf life of these cements and also their storage conditions[16].

$CaHPO_4$ (monetite) was reported[17,18] to have a very high aqueous solubility at and around physiological pH values compared to all other technologically important and biocompatible CaP phases, such as octacalcium phosphate [$Ca_8H_2(PO_4)_6 \cdot 5H_2O$], β-tricalcium phosphate and calcium hydroxyapatite. This high solubility has been the main stimulus for the current study, which aimed to prove the feasibility of forming the very first orthopedic cement based on $CaHPO_4$. Monetite was previously studied for applications in the treatment of dental caries and lesions as a constituent of chewing gums[19]. We have observed that (data not reported here) upon its immersion in a Synthetic Body Fluid (SBF) solution[20], $CaHPO_4$ converted into poorly-crystallized, carbonated apatitic calcium phosphate in a relatively short time (less than 48 hours at 37°C), which can be interpreted as an indicator of the highly promising bioactivity of this material. *In vitro* or *in vivo* biocompatibility of $CaHPO_4$ has not yet been reported, and we will soon publish the very first osteoblast cell culture (*in vitro*) results for this bioactive bone substitute material, in direct comparison to brushite, β-tricalcium phosphate, and hydroxyapatite.

To the best of our knowledge, a CaP cement which set into single-phase $CaHPO_4$ has not been reported yet. Such a $CaHPO_4$ cement scaffold was regarded to exhibit increased resorbability, both *in vitro* and *in vivo*, when compared to apatitic CaP cements.[21] Moreover, such inexpensive cement scaffolds may find a wider range of applications (beyond orthopedic uses) in the future as a class of "perfectly non-toxic and biocompatible" materials with easier formability. Our aim here was to synthesize monetite in cement form using a single, inexpensive, off-the-shelf powder component, i.e., calcium hydroxide ($Ca(OH)_2$), and phosphoric acid (H_3PO_4) as the major component of the setting solution.

EXPERIMENTAL PROCEDURE

Starting powder and setting solution

The powder component (*P*) of the cement consisted only of $Ca(OH)_2$ (>95%, Fisher Scientific, Fair Lawn, NJ), and it was used as-received, without any further treatments or additives. Two setting solutions (*SS*) were experimented with; setting solution-1 (*SS-1*) consisted of pure orthophosphoric acid (86.2%, H_3PO_4, Fisher Scientific, Fair Lawn, NJ). Setting solution-2 (*SS-2*) was prepared by mixing H_3PO_4, sodium hydrogen carbonate (>99.7%, $NaHCO_3$, Acros-Fisher Chemicals, Fair Lawn, NJ), and de-ionized water (DI, Millipore, 18.2 MW) in a glass beaker. For a typical preparation of SS-2, 1 g of $NaHCO_3$ was first placed in a 50 mL-capacity beaker and 3 mL of DI was added. This mixture was stirred with a glass rod for 2 minutes, followed by the slow addition of 12 mL of H_3PO_4 through a pipette or burette. Therefore, one was able to prepare 15 mL of the SS-2 at a time. Stable setting solutions were then stored at room temperature in tightly-capped glass media bottles.

Cement synthesis

For the cement synthesis, the setting solution *(SS-1 or SS-2)* was taken in a 10 mL-capacity glass vial and different amounts of DI were added to it to make *SS* with different volumetric proportions of DI. By this way, the amount of liquid (*L*) could be manipulated without changing the number of moles of P in the setting solution. The *SS* + *DI* (=*L*) solution was manually shaken in that small glass vial for 30 seconds. This liquid, *L*, was then added at once to the accurately weighed powder (*P*), i.e., $Ca(OH)_2$, which was previously placed into an agate mortar. The mixture was manually kneaded by using an agate pestle for a time at the end of which a paste-like substance could be easily scraped out of the mortar with the finger tips. By using different amounts of DI with the setting solution, the L/P ratio was easily changed. However, to obtain a consistent paste with good handling properties, 1.235 g of $Ca(OH)_2$ was placed in the mortar, 1.5 mL of *SS* was taken in the glass vial to which 0.4 mL of DI was then added. This optimized recipe corresponded to an L/P ratio of 1.54. The liquid was shaken for 30 seconds and then added to the powder, which after mixing for approximately 3 minutes yielded a good paste.

Characterization

Characterization of the starting powder i.e. $Ca(OH)_2$ included Fourier Transformed Infrared Spectroscopy (FT-IR, Nicolet 550, Thermo-Nicolet, Woburn, MA), scanning electron microscopy (SEM, FE-SEM, S-4700 and S-4800, Hitachi, Tokyo, Japan), and inductively coupled plasma atomic emission spectroscopy (ICP-AES, Model 61E, Thermo Jarrell Ash, Thermo Electron, Madison WI) analysis. Phase identification in set cement powder samples was perfomed by using powder X-ray diffraction (XDS-2000, Scintag, Sunnyvale, CA), operated at 40 kV and 30 mA, equipped with a Cu K_α-tube at a step size of 0.02° 2θ. FT-IR analyses were performed by Attenuated Total Reflection (ATR) method on powdered cement samples, using a diamond ATR window. Morphology of the cement samples was studied using SEM. Prior to the SEM studies, samples were coated with a thin layer of Pt to improve their conductivity. ICP-AES analysis was also used for the determination of Ca/P molar ratios in the set cement samples.

Compressive strength and setting time measurements

Cylindrical samples with an aspect ratio (length/diameter) of about 1.5 were prepared using a steel die. The formed cylinders were 18 ± 0.05 mm long, with a diameter of 12 ± 0.02 mm. To mention the procedure briefly, the paste was mixed in the agate mortar for a time that was required to get a good consistency depending on the L/P ratio studied. The paste was then manually forced into the cavity of the steel die, and a metal block with a weight corresponding to a pressure of only 0.775 MPa was placed on top of the mold for a period of 15 minutes. After 15 minutes, cylinders obtained were stored at 37±1°C for 36 h. Post incubation period, cylinder dimensions were measured using a digital caliper and compressive strength (CS) tests were carried out on a SATEC-Apex Universal testing machine by using a 4,500 kg load cell. A crosshead speed of 0.5 mm/min was used during the compressive loading runs.

A Gillmore needle apparatus was built and then used to measure the setting time of the cement. A weight of 453.6 g was used for the heavier stainless steel needle and 115.12 g for the lighter needle. t_I was the time in minutes when the lighter needle did not leave an indentation deeper than 1 mm on the cement surface and t_F was the time in minutes when the heavier needle failed to leave an indentation deeper than 1 mm on the cement surface.

RESULTS & DISCUSSION

Figure 1 showed the SEM pictures of the starting $Ca(OH)_2$ powders. As seen from these pictures, $Ca(OH)_2$ particles (particle size ranging from 2 to 10 μm) have a layered or stacked sheets-like microstructure. ICP analyses of the $Ca(OH)_2$ powders showed that the powders also contained 2600±150 ppm Mg. Magnesium is known to be an inhibitor of apatite crystallization,[22] and this proved to be an advantage about the selection of these powders for $CaHPO_4$-cement synthesis. Figure 2a depicted the IR spectra of the $Ca(OH)_2$ powders and it can be seen that there was some conversion of $Ca(OH)_2$ to $CaCO_3$ during storage at room temperature.

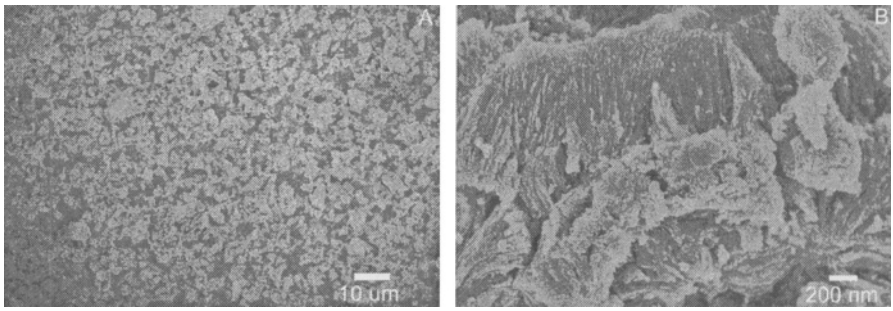

Fig. 1 (*a*) low magnification SEM image of the starting $Ca(OH)_2$ powders, and (*b*) a higher magnification image of the same

Preliminary experiments conducted by using SS-1, i.e., $H_3PO_4 + H_2O$ mixtures, led to the presence of monocalcium phosphate monohydrate (MCPM, $Ca(H_2PO_4)_2{\cdot}H_2O$) in all the samples. This was not surprising at all, high acidity in the cement pastes always led to the formation of more acidic calcium phosphates. The two IR spectra given in Figure 2b are obtained from samples prepared by using separate Ca/P molar ratios of 0.5 (top trace) and 0.9 (bottom trace).

An L/P ratio of 1.86 was used in both cases and the sample with a Ca/P molar ratio of 0.5 showed pure MCPM, while Ca/P molar ratio of 0.9 resulted in a more or less biphasic mixture of $CaHPO_4$ and MCPM. SEM analysis of these two samples also showed the characteristic large plate-like crystals of MCPM for the sample with Ca/P molar ratio 0.5 (shown in Figure 2c). The SEM image of sample with a Ca/P ratio of 0.9 showed two distinct phases, one growing as globules on top of the other, which is also confirmed by the FTIR spectra. Since the setting solution contained only H_3PO_4, the pH value of the system went down to a very low value, hence the observation of MCPM was expected.[23] Moreover, since MCPM had a very high rate of nucleation under the specific synthesis conditions we used, the materials obtained did not show any extent of self-setting cement characteristics and turned into a hard mass very rapidly in the mortar.

The amount of MCPM obtained in the final mixtures decreased in going from the Ca/P molar ratio of 0.5 to that of 0.9, and since the theoretical Ca/P molar ratio of the aimed stoichiometry (monetite, $CaHPO_4$) was 1.0, further experiments were performed with a Ca/P ratio of 0.9. Effect of different L/P ratios on the final phase composition was also studied using SS-1, and MCPM was found to be present in all samples at all L/P ratios although the amount of MCPM varied; a higher L/P ratio led to decreased MCPM amounts in the final material. It can be hypothesized that the fluid pastes obtained by using higher L/P ratios would have higher pH values compared to those obtained by using comparatively lower L/P ratios. This slightly higher pH combined with better mixing due to increased amounts of DI could be one reason for the observation of decreasing amounts of MCPM at higher L/P ratios.

MCPM is an extremely acidic phase and for that reason it can neither be desirable nor used alone as a biomaterial for skeletal repair. Highly acidic calcium phosphates, such as MCPM, cause immediate inflammatory response upon implantation. On the other hand, the inflammatory response to brushitic cements was found to be quite mild and transient in nature.[13] Since the very low pH of the setting solution was the sole reason for MCPM formation, an additive which would increase the pH of the setting solution only slightly, such as $NaHCO_3$, was regarded to alleviate the problem of MCPM formation in the cement. Different amounts of $NaHCO_3$ in a new setting solution (i.e., SS-2) were experimented with, and a very small amount of $NaHCO_3$ addition (0.066 g/mL) to the setting solution (as described in the Experimental section) was found to be extremely effective in completely eliminating the MCPM phase from the final set cements as seen from the IR and XRD data given in Figures 3a and 3b, respectively.

The development of the new SS-2, therefore, resulted in obtaining pure $CaHPO_4$ as the final product of the set cements. ICP analyses of the set cements gave the Ca and P wt% values of 24.91 and 19.86, respectively. This translated to a Ca/P molar ratio of 0.96 in the solid cement samples, which was in close agreement with the theoretical Ca/P molar ratio for $CaHPO_4$ (1.0). With a further increase in the concentration of $NaHCO_3$ in the setting solution (i.e., SS-2 derivatives), apatitic CaP formation was seen along with monetite (results not shown). Some unreacted $Ca(OH)_2$ was also observed in the XRD plot, which did not readily show up in the IR spectra.

SEM photomicrographs of Figures 3c through 3e showed the microstructure of the set cement samples. At a low magnification (Fig. 3c), the cement was seen to contain a significant amount of pores which were interconnected (observed at higher magnifications). Porous cements are favorably required to allow for the wicking of blood and blood cells towards the bulk of the cement samples. At higher magnifications (Figs. 3d and 3e), the SEM images also showed that the cement microstructure was quite different from that of the starting powder, i.e., $Ca(OH)_2$.

Interlocking of $CaHPO_4$ plates was also seen at numerous places which imparted the cement its limited but still significant strength.

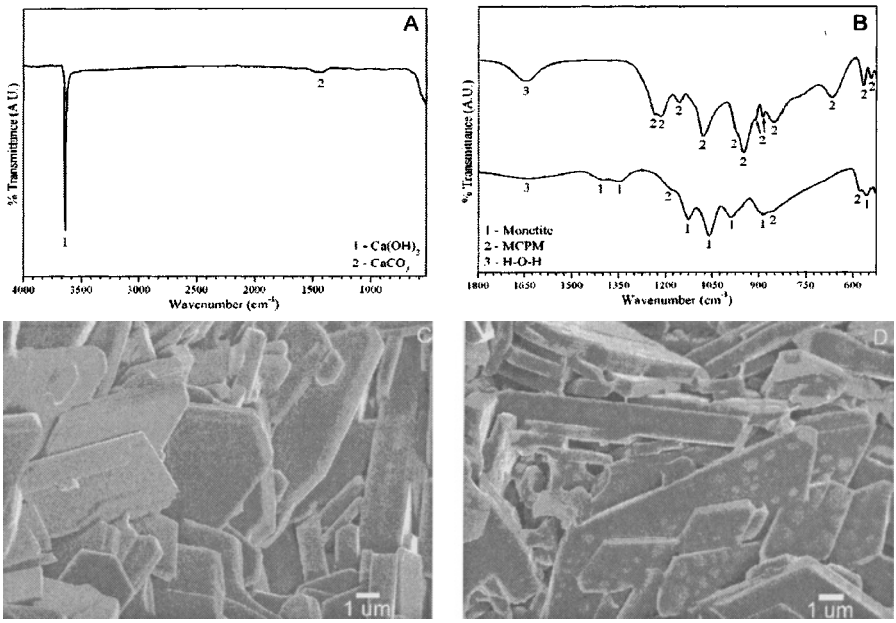

Fig. 2 (*a*) IR spectrum of starting Ca(OH)2 powders, (*b*) bottom: IR spectrum of Ca/P=0.5, SS-1 substance, and top: IR data for Ca/P=0.9, SS-1 substance, (*c*) SEM image of sample obtained with a Ca/P ratio of 0.5, SS-1, MCPM crystals, (*d*) SEM image of a Ca/P ratio of 0.9, SS-1 substance, MCPM crystals together with a small amount of $CaHPO_4$.

The mechanical strength of any orthopedic cement is an important parameter since the cement should have some load-bearing ability during its lifetime as an implant. Figure 3f showed a characteristic compressive strength (CS) chart for the $CaHPO_4$ cement of this study. The CS of the cement was found to be 2.05 ± 0.2 MPa. It should be noted that the human trabecular bones have a modest compressive strength over the range of 2 to 10 MPa.[1,2] The setting time of the cement of this study was determined by using the Gillmore needle apparatus and the optimized cement recipe had an initial setting time, i.e., t_I, of 19 ± 2 minutes and a final setting time, t_F, of 58 ± 3 at an L/P ratio of 1.54.

The rate of nucleation of monetite (with SS-2) was somewhat slower in comparison to the highly acidic MCPM (with SS-1), therefore, the reaction mixture showed a self-setting property as free Ca^{2+}, $H_2PO_4^-$, and HPO_4^{2-} ions were gradually consumed, and the growth of monetite crystals took place according to the tentative reaction:

$$Ca(OH)_2 + H_3PO_4 + Na^+(aq) + HCO_3^-(aq) \rightarrow CaHPO_4 + 2H_2O + Na^+(aq) + HCO_3^-(aq) \quad (1).$$

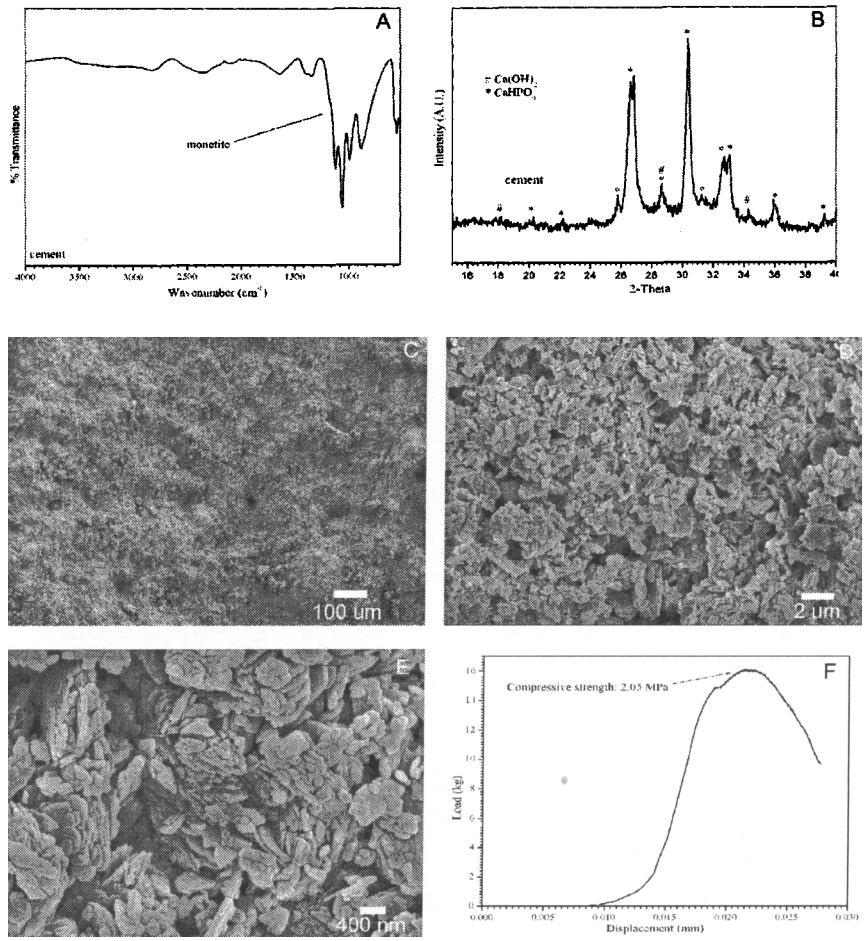

Fig. 3 (*a*) IR spectra of $CaHPO_4$ cement, (*b*) XRD data of $CaHPO_4$ cement, (*c*), (*d*) and (*e*) SEM images of $CaHPO_4$ cement, (*f*) compressive strength data of $CaHPO_4$ cement

Although the above reaction showed the use of H_3PO_4 in the reactants, the setting solution (SS-2) which gave rise to the synthesis of this inexpensive and low-strength $CaHPO_4$ cement presumably contained a mixture of $H_2PO_4^-$ and HPO_4^{2-} ions, together with some hydrogen carbonate ions. MCPM is known to be the stable phase at a pH below 2.5, whereas monetite ($CaHPO_4$) becomes the stable phase over the pH range of 2.5-4.2[23].

The addition of a small amount of $NaHCO_3$ to the H_3PO_4-based setting solution was quite influential in bringing the pH of the cement paste down to a range where monetite became the most favored phase, and this has been the main idea behind this very simple cement. In vitro testing of these cements is in progress.

CONCLUSIONS

A very simple, inexpensive and a robust technique for the preparation of a cement consisting of single phase monetite ($CaHPO_4$) was presented. $Ca(OH)_2$ was reacted with an aqueous mixture of H_3PO_4 and $NaHCO_3$ to form $CaHPO_4$ in solid, cement form. Since the final product of the cement was only $CaHPO_4$ of high aqueous solubility, the cement bodies are also regarded to have high bioresorbability in comparison to apatitic cements. The cement pastes showed very good handling properties and possessed a setting time less than 20 minutes.

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