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Systematic Assessment of Synthesized Tri-magnesium Phosphate Powders (Amorphous, Semi-crystalline and Crystalline) and Cements for Ceramic Bone Cement Applications

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Magnesium phosphate cements have come under investigation in recent years for use as an alternative to calcium phosphate cements for bone void repair applications. Evidence indicates that magnesium phosphate cements obtain higher initial strengths after cement reaction and resorption in more clinically appropriate time frames than commercially available calcium phosphate cements. In this study, amorphous, partially amorphous and crystalline tri-magnesium phosphate powders were synthesized via an aqueous precipitation reaction with subsequent thermal treatment, and characterized using techniques such as X-ray diffraction and Fourier transform infrared spectroscopy. These materials were assessed for their functionality in cementing reaction with a 3.0 mol/L, pH 7.0 ammonium phosphate solution, including setting time and pH evolution in phosphate buffered saline solution. Results indicated that the amorphous and semi-crystalline tri-magnesium phosphate powders were highly reactive with the setting solution but resulted in mechanically weak cements, while the crystalline tri-magnesium phosphate powder reacted efficiently with the cement solution and were mechanically strong following the cement reaction. X-ray diffraction and scanning electron microscopy analyses indicated significant changes in the phase composition and morphology of the cements following incubation in phosphate buffered saline. These were perceived to be detrimental to the integrity of the amorphous and semi-crystalline tri-magnesium phosphate derived cements but not to those created with fully crystalline tri-magnesium phosphate. The crystalline tri-magnesium phosphate material resulted in the most functional cement as this embodiment displayed the most clinically relevant setting time as well as the highest mechanical resilience and neutral pH during incubation in saline solution rendering them potentially viable as bone void fillers.

1. Introduction

Bone substitutes are widely used in patients who require implantation to repair or remodel bone defects. These bone substitutes can range from synthetic materials such as metals and polymers to natural polymeric and biologic materials including allografts and autografts[1,2]. Calcium phosphate based synthetic grafts are an excellent choice of bone replacement systems because these implants mimic the chemistry of the mineralized portion of human bone and have shown excellent biocompatibility[3,4]. Currently, there are calcium phosphate based bone cements clinically available, however these products are less than ideal. Optimal bone cements should display high biocompatibility and osteoconductivity, strengths similar to natural bone, resorption rates in line with rapid bone remodeling, and clinically appropriate mixability, injectability, and setting times[4–6]. Brushite ($CaHPO_4$) based calcium phosphate bone cements, although readily resorbable, tend to display prohibitively fast setting rates and compressive strengths lower than natural bone. Hydroxyapatite ($Ca_5(PO_4)_3(OH)$) based calcium phosphate bone cements are capable of achieving

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higher strengths, but set very slowly and require several years to resorb and allow for bone remodeling[3,7].

Recently, it has been proposed that magnesium phosphate cements may pose a more clinically favorable option to traditional calcium phosphate cements[8]. Preliminary studies have shown that magnesium phosphate based cements display higher strengths, better setting time and faster resorption rates than calcium phosphates, while maintaining a high level of biocompatibility[8–16]. However, in contrast to calcium phosphates, the intensity and depth of study into these cement systems is still limited to only a few phases and remains to a large extent unexplored with inconsistent evaluation. The proposed study is to explore the cementing characteristics of amorphous and crystalline tri-magnesium phosphate (TMP), $Mg_3(PO_4)_2$ ceramic. Trimagnesium phosphate is a potential bone cement precursor material critical to the cementing reaction. It is important to study the precursor reactants as they correlate to the final cement product since the material properties affect the reaction kinetics, cement formulation and final product properties.

The study of magnesium phosphates for bone cements is a relatively new field of study compared to traditional calcium phosphates. Calcium phosphate bone cements have been exploited to a large extent because the composition closely mimics mineralized bone matrix, however mechanical strength and resorption rate of these cements are not ideal. In addition to calcium, many other ions such as magnesium, silicon, zinc, potassium and copper play important roles in bone regeneration, from regulating the metabolic processes and osteoblastic differentiation genes to promoting angiogenesis and increasing bone cell adhesion[17,18]. With regards to bone formation and dissolution, magnesium has been shown to play a major role in calcification and bone density, mineral metabolism, hydroxyapatite crystal formation and increased bone cell adhesion and stability[16,19–21]. Phosphate is not only a main component of bone growth, but has also been shown to stimulate expression of matrix gla protein which is involved in bone formation[17]. However, high concentrations of any of these ions, including calcium, can significantly impede the viability of cells[22,23]. Recent studies into magnesium phosphates have been conducted due to higher strengths and faster resorption rates of these cements. Magnesium phosphate cement or ceramic compositions explored to date include $MgHPO_4 \cdot 3H_2O$, $MgKPO_4 \cdot 6H_2O$, $Mg_3(PO_4)_2 \cdot 8H_2O$, $MgNH_4PO_4 \cdot 6H_2O$, and $Ca_3Mg_3(PO_4)_4$[8,24–28].

The use of amorphous calcium phosphates (ACPs) in bone scaffolds and cements is known, however the level of understanding regarding the potential of amorphous magnesium phosphates for biomedical applications is still very marginal. ACPs are often regarded as highly useful as scaffolds or scaffold precursors because of the highly reactive and soluble nature of the ACP[29]. ACP is a critical part of the natural bone formation process, a transient phase which is a precursor to hydroxyapatite (HA)[30–32]. These ACPs have been used in bone cements to increase the cement reactivity or bioresorbability. ACPs have also been shown to increase osteoconductivity of implant coatings as well as increase the mechanical properties of composites[30]. Magnesium has been widely shown to stabilize calcium phosphate in the ACP stage over conversion to HA[33,34]. However, the analogy between ACP and amorphous magnesium phosphate (AMP) is not clear as the synthesis of amorphous magnesium phosphate has only recently been reported[35]. In a recent study from our laboratory, a comparison of the cytocompatibility of dense substrates of amorphous magnesium phosphate and crystalline tri-magnesium phosphate showed increased proliferation and differentiation of murine pre-osteoblast cells on the amorphous substrates over the crystalline substrates[36]. It is however unclear whether the use of amorphous tri-magnesium phosphates (ATMPs) is feasible to generate a functional bone cement product, or more importantly, how this cement may compare to cements made with a crystalline tri-magnesium phosphate. In this study, the functionality of these amorphous and crystalline tri-magnesium phosphate powders, as well as semi-crystalline tri-magnesium phosphates powders, in cement forming reactions with a concentrated, neutral pH ammonium phosphate solution for bone cement applications is explored and systematically assessed.

2. Experimental

2.1. Magnesium phosphate powder synthesis

Magnesium phosphate powders were synthesized via an aqueous precipitation reaction between 0.3 mol/L $MgCl_2$ and 0.2 mol/L Na_3PO_4, in deionized water. The magnesium containing solution was pipetted into the phosphate containing solution, resulting in the precipitation of tri-magnesium phosphate (TMP), $Mg_3(PO_4)_2$. The pH of the reacting solution was observed to be basic and was not controlled during the synthesis. The solution was centrifuged at 10,000 r/min for 5 min and the excess liquid was discarded. The TMP precipitate was collected and rinsed thrice in deionized water followed by washing in acetone and finally dried overnight at 60 °C. Powders were then thermally treated at 400, 600 or 800 °C for 6 h at each temperature in a box furnace in air to form amorphous, semi-crystalline or fully crystalline dehydrated TMP, respectively. Powders were lightly ground and sieved to −500 μm to remove any large agglomerated clusters.

2.2. Magnesium phosphate powder characterization

Magnesium phosphate as-synthesized and thermally treated powder samples were characterized utilizing a number of techniques. Thermogravimetric analysis and differential scanning calorimetry (TGA and DSC) were used to identify the level of hydration and temperature of crystallization utilizing a Netzsch STA 409 PC Luxx system. The as-synthesized powder was heated at a rate of 10 °C/min to 1000 °C under argon. Crystalline phases were identified by X-ray diffraction (XRD) using a Philips X-Pret PRO diffractometer equipped with Cu$K\alpha$ radiation and X'celerator Si-detector. The X-ray generator was operated at 45 kV and 40 mA at a 2θ range of 10°–90°. Fourier transform infrared spectroscopy (FTIR) was completed on a Nicolet 6700 spectrophotometer with a diamond ATR Smart orbit. FTIR spectroscopy was used to identify the differences in molecular chemical linkages between amorphous and crystalline precipitates. Spectra were collected at 1.0 cm^{-1} resolution averaging 64 scans in the 400–4000 cm^{-1} frequency range. Specific surface area (SSA) measurements was determined from the N_2 adsorption–desorption isotherms obtained using the Brunauer–Emmett–Teller (BET) method performed on a Micromeritics ASAP 2020 instrument. SSA measurements were used to determine the area, and subsequent relative reactivity of synthesized powders. Particles were observed by scanning electron microscopy (SEM) using a JEOL, JSM-6610LV system. Samples were sputter coated with palladium on a Cressington sputter coater 108A prior to imaging.

2.3. Cement formation

Series of cements were compared by combining the amorphous, semi-crystalline and crystalline $Mg_3(PO_4)_2$ powders with a pH 7.0, 3 mol/L solution of ammonium phosphate. The solution was made by combining monobasic ammonium phosphate ($NH_4H_2PO_4$) and dibasic ammonium phosphate ($(NH_4)_2HPO_4$) salts in deionized water at a ratio to obtain pH 7.0. The ratio of monobasic to dibasic