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Preparation of micro-porous bioceramic containing silicon-substituted hydroxyapatite and beta-tricalcium phosphate

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ABSTRACT

Dimensional instability caused by sintering shrinkage is an inevitable drawback for conventional processing of hydroxyapatite (HA). A new preparation method for biphasic calcium phosphates was developed to increase micro pores and biodegradation without significant dimensional change. Powder pressed HA discs, under 100 MPa, were immersed in a colloidal mixture of tetraethoxysilane (TEOS) and ammonium hydroxide for 10 min, followed by drying, and then were sintered at 900 °C, 1050 °C, and 1200 °C, respectively. Comparing with pure HA discs, the newly prepared product sintered up to 1200 °C contained silicon substituted HA, beta-tricalcium phosphate, and calcium silicate with better micro-porosity, high specific surface area, less sintering shrinkage and the strength maintained. The cytocompatibility test demonstrated a better viability for D1 mice stem cells cultured on TEOS treated HA for 14 days compared to the pure HA. This simple TEOS sol-gel pretreatment has the potential to be applied to any existing manufacturing process of HA scaffold for better control of sintering shrinkage, create micropores, and increase biodegradation.

1. Introduction

Bone grafting is usually performed for patients with severe periodontal disease, traumatic injury or tumor ablation on bone tissue and filling or reconstruction of the osseous defects required. Autogenous bone (taken from another parts of the patient) is one of the most commonly utilized material for grafts, however, new defects are created at the harvest site with the use of autogenous bone which have been associated with a 17.9–26.6% major complication rates, including morbidity of the donor site [1–3]. It was for these reasons that biodegradable artificial bone grafts have been proposed and gained significant traction as a plausible alternative to autogenous bone grafts in the recent decades.

Because of the superior osseoconductivity as well as the lack of virus infection and allergic risks, calcium phosphates such as hydroxyapatite (HA), beta-tricalcium phosphate (β-TCP) and their composites have all been trialed as favorable alternatives for bone grafting in dental, maxillofacial, and orthopedic clinical practice. For an ideal graft material, it must comprise sufficient integral strength to allow the successful implantation of grafts as well as controllable degradation which is crucial for bone regeneration [4]. Modification of phase structures is one of the most common methods employed to control in-vivo degradation. More specifically, the highly crystalline HA possess limited dissolution in body pH environment. In contrast, β-TCP has a much higher dissolution rate, though the β-TCP does not dissolve in body fluids at physiological pH levels, dissolution of β-TCP requires cell activity producing acidic pH [5]. Thus, adjusting the ratio of biphasic calcium phosphates (usually obtained with HA and β-TCP in different ratios) can obtain a more appropriate degradation. Furthermore, porosity is also a key factor for adjusting the degradation time and to facilitate the bone ingrowth [6,7].

The degradability of HA/β-TCP has also been reported to be improved by doping ions in scaffolds or mixing start powders with bioactive glass [8,9]. Dopants such as silicon, zinc, strontium and magnesium in HA/β-TCP scaffolds have the ability not only to better control the dissolution rates, change densification behavior, and improve mechanical strength but have also been reported to enhance the biocompatibility [9–12]. Doping ions in HA, such as Si^{4+}, Mg^{2+}, and F^{-} often causes a decrease in grain size and subsequently affects dissolution rates and osteoblast cells activities [13–18]. Among these studies, silicon (Si) substitution in the bioceramic of calcium phosphates has been the most discussed and demonstrated great potentials.

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Silicon is an essential element for bone formation and calcification. The silicon concentration was found to be 0.5 wt% in mineralizing osteoid regions in young mice and rats, which are 25 times greater than the surrounding areas and the silicon content gradually declined as calcification occurs [19]. Doping of Si into HA can generally improve the bioactivity of HA. Gibson et al. demonstrated that Si substitution in HA (Si-HA) enhances osteoblast cell activity when compared to pure HA phase [20]. The same positive outcomes have been reported in animal studies, where rapid remodeling of bone surrounding the Si-HA and greater rates of bone apposition were found when comparing against pure HA [18,21]. It has been identified in-vivo studies that Si changes the grain size, topography [22,23], chemical composition [20], crystallinity [24], and surface charges [3] that enhances the formation of apatites on the Si-HA surface [21,22,25]. It is suggested that high in-vivo degradation rate [18] and appropriate silicon ion release [26] all contributed to the cell affinity and bone tissue regeneration. And 4 mM Si ion in culture medium has been identified to increase the type 1 collagen gene expression and extracellular signal regulated kinases secretion of osteoblasts that positively regulates angiogenesis, proliferation, differentiation, and morphogenesis [26].

Attention has recently been drawn to the mechanical properties of Si-doped calcium phosphates. Bang et al. synthesized three different compositions of 0.4, 0.8 and 1.6 wt% Si to form Si-HA powders and sintered at 1150, 1200 and 1250 °C. It was found that the diametral tensile strength (DTS) of Si-HA increases with increasing Si contents and becomes much higher than that of pure HA [27]. Using drying quartz as additive in original powder, Kanazawa et al. also found that the mechanical properties of sintered porous calcium phosphate ceramics, such as TCP or HA, can be improved by the doping of SiO_2 [28]. Bandyopadhyay et al. found that doping with SrO/SiO_2 reduced compressive strength of the β-TCP samples to 57% of pure β-TCP. The impact of dopants on long-term in vitro strength degradation was evaluated by soaking in simulated body fluid (SBF) for a period of 8 weeks. Their results showed that most ion doped β-TCP have less in-vitro degradation in simulated body fluid (SBF) and doping with SrO/SiO_2 was more prone to apatite formation and lead to weight increase [29]. Fielding et al. used 0.5 wt% SiO_2/0.25 wt% ZnO as sintering additives for 3D printing fabrication of β-TCP scaffold, demonstrating a 2.5 fold increase in compressive strength by liquid phase sintering [30]. Velasquez et al. used a powder metallurgy method (under 200 MPa and sintered at 1500 °C) to produce α-TCP ceramics doped with either 0, 1.5, or 3.0 wt% of dicalcium silicate. Overall, the in-vitro and in-vivo results showed that the Si doped TCP had best bioactive properties at 3.0 wt% compositions and with a slight enhance in mechanical strength [31]. Recently, Manchón et al. demonstrated a synthetic bone graft composed of β-TCP and silicocarnotite (calcium phosphate silicate mineral) through solid state reaction of DCPD and fumed SiO_2 producing complete degradation and efficient enhancement of bone formation in animal study [32]. Most Si doped calcium phosphates in these studies are produced by sol-gel forming nano-sized powder or by mixing glass powder with calcium phosphates, then compacted and sintered to their final application form. Despite the promising results of silicon substituted calcium phosphates, few studies have yet specifically investigated the consequential effect of the additional micro porous on its mechanical properties.

The present study investigated the characteristics of biphasic calcium phosphates with micropores prepared by immersing hydroxyapatite compact disc in tetraethoxysilane (TEOS) followed by triggering hydrolysis and condensation reaction to allow silicon gel formation before final sintering. The silicon gel is anticipated to enhance the compact of powder and react with the HA particles through solid state diffusion at high temperature. The effects of TEOS sol-gel pretreatment on disc strength, porosity, composition, phase structure and degradation were evaluated and documented.

2. Methods

2.1. Sample preparation

Four hundred milligrams of HA powder (Lot# SZBC0740V, Sigma-04238, SIGMA-ALDRICH) were uniaxially pressed into compact at 100 MPa (Model 3851, Carver, USA) for 3 min in two different molds (diameter of 14 mm for biaxial flexural strength test or 12 mm for all other evaluations). The sol-gel process was performed by using tetraethyl orthosilicate (TEOS, $Si(OC_2H_5)_4$, 98% purity, SIGMA-ALDRICH) as a precursor in a fixed Ethanol:TEOS:H_2O ratio of 40 mL:25 mL:15 mL (0.685 mol:0.112 mol:0.833 mol). For the hydrolysis and condensation reactions, the pH value of sol-gel solution was adjusted to 11 by added ammonium hydroxide as a catalyst. The HA compacts were immersed in the solutions (10 mL per disc) for 10 min under vacuum. The samples were rested in room temperature for 1 h and 2 more hours in 60 °C oven for condensation and to dry, then the samples were sintered at a ramp rate of 15 °C/min to 900, 1050, and 1200 °C for 5 h in air (giving sample code T900, T1050, and T1200 respectively). The same samples without TEOS sol-gel pretreatment were set as controls; giving code H900, H1050, and H1200 respectively.

2.2. Biaxial flexural strength test

Sample discs of 1.2 mm in thickness and 14.0 mm in diameter were prepared as previous sol-gel and sinter procedures for biaxial flexural strength test. Specimens were tested using a universal testing machine (JSV-H1000, Japan Instrumentation System, Nara, Japan) with a cross-head speed of 1.0 mm/min using a pin on three ball configuration. Mean biaxial flexural strengths were calculated following equation (Eq. 1) and subjected to statistical analysis followed an ISO standard (ISO 6872). Fig. 1 presents the schematic diagram of testing setup.

$$\sigma = \frac{-0.238F(X-Y)}{d^2}$$
$$X = (1+\nu)\ln\left(\frac{r_2}{r_3}\right)^2 + \left[\frac{(1-\nu)}{2}\right]\left(\frac{r_2}{r_3}\right)^2 \quad (1)$$
$$Y = (1+\nu)\left[1 + \ln\left(\frac{r_1}{r_3}\right)^2\right] + (1-\nu)\left(\frac{r_1}{r_3}\right)^2$$

The F is the total load causing fracture (N), ν is Poisson's ratio (0.25), r_1 is the radius of the support circle (10.0 mm), r_2 is the radius of the loaded area (1.5 mm), r_3 is the radius of the specimen (14.0 mm), d is the specimen thickness at the origin of fracture (1.2 mm).

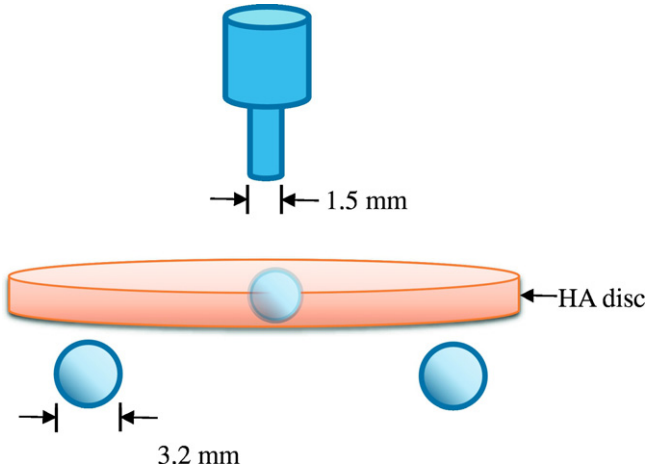

Fig. 1. Schematic diagram of the testing setup for biaxial flexural strength.