Three-dimensional fully interconnected highly porous hydroxyapatite scaffolds derived from particle-stabilized emulsions

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Abstract

Three-dimensional (3D) macroporous hydroxyapatite (HAp) ceramics were fabricated via a facile emulsion templating technique for bone tissue engineering applications. The prepared scaffolds have an open, uniform, and 3D fully interconnected pore structure with high porosities, mechanical strength, and a large surface/volume ratio. The porous structure, which is composed of an array of quasi-spherical large cells interconnected through windows, has a pore diameter in the range of 5–30 μm. The samples sintered at the optimal temperature (1200 °C) have an open porosity of ~76.5% and a high compressive strength of 4.26 MPa. The technique discussed here is a significant advancement over other previously used methods to produce HAp ceramics and is expected to enable much improvement in the topology of the porous structure of scaffolds.

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1. Introduction

Hydroxyapatite (HAp) ceramics have been widely studied and extensively used in clinical applications over the last few decades owing to their excellent biocompatibility and similarity in composition with the natural bone [1–3]. Porous HAp scaffolds, in particular, have attracted much attention since they exhibit high osteoconductivity and bioactivity; these properties are very useful for hard tissue applications [4–6]. From the viewpoint of osseointegration and osteoconductivity, it is desirable to develop scaffolds with high porosities and spherical, open, and interconnected pores [7–9]. These bioactive porous HAp ceramics can be colonized by bone tissue with the same characteristics as those of peri-implanted tissues [10]. A variety of manufacturing methods have been extensively reviewed and developed to fabricate porous HAp scaffolds; these typically include replication [11], sacrificial templating [12,13], tape/gel casting [14,15], and a direct foaming technique [16,17]. These methods have many advantages such as their safety and easy reproducibility; they also allow the production of materials with high porosity and complex shapes; however, these methods usually result in either poorly interconnected, closed, and non-uniform pores, or poor mechanical strength for load-bearing applications.

Previous studies have investigated implants with random pore geometries that range widely in porosity and size. In these studies, a pore size greater than 150 μm was shown to be an essential requirement for osteoconduction [18,19]. However, recent clinical evidence has revealed that a 3D interconnected porous structure, which ensures cell attachment and flow of nutrients, is the key factor governing osteoconduction rather than the size of the pores themselves [20–23].

Therefore, the preparation of highly porous HAp ceramics with 3D interconnected spherical pores has been the subject of many studies. The preferred method of fabricating such bioceramics should allow for tailoring of their porosity (offering 3D interconnections) and achieving of solid ceramic networks. The Pickering high internal phase emulsion (HIPE) templating technique, which involves the preparation of...
emulsions with inorganic particles in the continuous phase and subsequent removal of the dispersed droplets by drying and heat treatment, has recently attracted considerable attention for the manufacture of high porosity ceramics. This versatile method provides control over the resulting porous structure and yields ceramics with a highly ordered microstructure and enhanced mechanical properties [24–26]. In this paper, particle-stabilized emulsions were used as the templates, and a facile method for preparing high-porosity HAp ceramics with solid 3D interconnected spherical pores has been reported.

2. Materials and methods

Ceramic suspensions were prepared by adding commercially available nano HAp powders (d50 = 60 nm, Nanjing Emperor Nano Material Co. Ltd.) stepwise to deionized water containing 0.5 wt% of the ammonium salt of poly (acrylic acid) (Adamas Reagent Co., Ltd.) and keeping the suspension pH at 11.0. The pH was adjusted by using ammonia (4 M, Sinopharm Chemical Reagent Beijing Co., Ltd.) and hydrochloric acid (2 M, Sinopharm Chemical Reagent Beijing Co., Ltd.). The amount of solid loaded in the suspension was initially fixed at 25 vol%. Homogenization and deagglomeration were carried out in a ball mill for at least 24 h. The pH was readjusted to 11.0 after the completion of the ball-milling process. Then, a certain amount of propyl gallate (1.5 wt% to HAp powders) was dissolved in ethanol (AR, Beijing Chemical Works) and added dropwise to the ball-milled suspension to modify the HAp particles in situ [27]. Next, 200 vol% of octane (AR, Beijing Chemical Works) was added to the suspension as the oil phase, and particle-stabilized emulsions were obtained by mechanical shearing of the suspensions using a household mixer at full power for 5 min. The emulsions exhibited remarkable stability against coalescence, drainage, and creaming because of the adsorption of surface-modified particles at the freshly created oil/water interface. All the emulsions were placed in a culture vessel (2 cm in height and 9 cm in diameter) and left to dry at room temperature for one week to allow the oil and water to evaporate. After the emulsions had dried, the samples were shaped. Finally, the samples were sintered at 1100–1400 °C for 3 h; the ramp rate from room temperature was 3 °C min⁻¹. The experimental procedure is illustrated in Fig. 1.

The commercial HAp powders and sintered specimens were analyzed by X-ray diffraction (XRD, D’Max-Ra12kW, Cu-Kα radiation, Ouyatu, Japan) and Fourier transform infrared spectroscopy (FT-IR, FTIR-8400, Shimadzu, Japan) in order to verify whether the high temperature heat treatment destroys the phases present and surface structure of the HAp. The morphologies of the samples were investigated by scanning electron microscopy (SEM, S-4800, Hitachi, Japan). The porosity of the specimens was determined by mercury porosimetry (Pore Master GT60, Quantachrome). The dimensions and weights of the sintered samples were measured to determine the bulk densities and porosities. The compressive strengths of the sintered samples (radius/height = 1.5) were measured using a Universal Testing Machine (ANS, MTS Systems (China) Co., Ltd., China) at room temperature with a push speed of 0.1 mm/min.

3. Results and discussion

Fig. 2a shows the XRD patterns of the commercial HAp powder and the ceramic film after being sintered at 1200 °C for 3 h. The XRD peaks of the two diffraction patterns agree well with those of standard HAp in the powder diffraction file (JCPDS9–432). Compared with the XRD pattern of commercial HAp powder, no apparent decomposition of HAp and additional phases can be identified in the XRD pattern of sintered porous HAp ceramics; the more resolved XRD peaks of the sintered samples indicate growth of the HAp crystals. This indicates that the sintering process did not alter the composition of HAp and can thus allow the formation of a fully densified structure at high sintering temperatures.

The FT-IR spectrums of the commercial HAp powder and the sintered porous HAp ceramic are shown in Fig. 2b. In this spectrum, the absorptions at 962 cm⁻¹ (ν₁ of PO₄), 1044 and 1090 cm⁻¹ (ν₃ of PO₄), and 571 and 602 cm⁻¹ (ν₄ of PO₄) are...
typical for apatite. The two bands at 3571 cm\(^{-1}\) and near 632 cm\(^{-1}\), corresponding to the vibration of the OH\(^{-}\) groups in HAp, can also be seen. There is no discernible difference between the spectrum of the commercial HAp powder and that of the porous HAp ceramic, except for the bands at 1419 and 1448 cm\(^{-1}\), which are indicative of the carbonate ion substitution, and the bands at 1643 and 3398 cm\(^{-1}\), which correspond to the disappearance of absorbed water after sintering [20,21]. These results further confirm that no chemical decomposition occurred during the sintering process.

Bioactive scaffolds are required to reproduce bone characteristics not only in terms of composition but also in terms of pore morphology. Although there is no single, widely accepted definition for optimal pore sizes of biomaterials for osteoconduction and bone ingrowth [28]. It is generally accepted that the pore size of 100–300 μm is necessary to provide the space for notable cells grow into and osteoconduction [29,30]. However, a number of studies have found that microporosity and pore wall roughness also play an important role for osteogenic outcomes [31–33]. It was found that pores of 20–50 μm diameter might give a favorable function for physiological liquid exchange, nutrition transportation and bone-like apatite formation by dissolution and reprecipitation [34,35]. Moreover, small pores (pore size < 10 μm) can results in larger surface area that is believed to favor hypoxic conditions and protein adsorption [36]. Therefore, developing a versatile method to prepare porous bioceramics with far-ranging pore diameters and to fabricate porous bio-scaffold with a porosity gradient biomodal structure is highly desirable. A representative cross-sectional microstructure of the porous HAp ceramics fabricated from particle-stabilized emulsions is shown in Fig. 3. Low-magnification images of the resulting material reveal that the extended network is dominated by quasi-spherical large cells with open windows in the lamella walls. At higher SEM magnification, we observe a
3D fully interconnected ordered structure. The final products have uniformly distributed macropores with diameters ranging from 5 to 30 μm. A large number of windows were distributed on the walls of these macropores, resulting in large voids between matrix grains.

It is well known in the fields of bone regeneration and tissue engineering that scaffold architecture is critical to allowing biomolecules and degraded substances to flow freely into and out of the scaffold. Thus, pores that are fully interconnected are more likely to induce bone ingrowth [1,2]. Yoshikawa and Myoui [23] found that natural bone ingrowth occurred in all pores after a certain time had elapsed following the implantation of a fully interconnected porous HAp bioceramic. Fig. 4 shows the pore volume and distribution of the sintered (1200 °C) porous HAp ceramic. The inset table summarizes the data obtained. The distribution is unimodal with a peak at a pore diameter of ~4 μm. The total open porosity of the samples is ~81.4%, and the average pore size is approximately 3.91 μm. The average pore size is much smaller than those of the cavities observed by SEM (Fig. 3); this is owing to a “bottle neck” effect that occurs when mercury enters into a large cavity through a narrow channel [37]. The sizes determined by mercury porosimetry therefore correspond to the pore throat diameters and are thereby consistent with the SEM results. As for the prepared highly porous HAp scaffolds, they can provide a pathway for bone attachment and new bone phase ingrowth owing to their large surface area. The 3D ordered pore structures with interconnected macropores can act as channels for the transport of bodily fluids, thus resulting in scaffold materials that may have excellent bioactivity and osteoconductive properties [9,38].

It is well known that highly porous structures are advantageous to osteoconduction and bone ingrowth. However, the mechanical strength of a material generally decreases as its porosity increases. A balance between these two factors should help achieve a compromise between biological and mechanical requirements of HAp ceramics for clinical load-bearing bone tissue engineering applications [10,21]. Fig. 5 shows the effects of sintering temperature (1100–1400 °C) on the porosity and compressive strength of the porous HAp ceramics. With increase in the sintering temperature from 1100 to 1400 °C, the porosities decrease from 84.2% to 66.4%, while the bending strength increases from 0.72 to 10.2 MPa and the compressive strength from 1.18 to 14.6 MPa. For samples sintered at 1200 °C for 3 h, the high macroporosities (~76.5%) and compressive strength (4.26 MPa) obtained represent a good balance for BTE applications. It is noteworthy that the compressive strength for porous HAp ceramics developed in this work is much higher than those with similar porosities [1–3]. It is thus reasonable to expect that the mechanical property of such HAp ceramics, together with the 3D interconnected pore structure, will significantly enhance the performance of bone repair in clinical applications.

Fig. 6 shows the SEM images of fracture surface of the porous HAp ceramics sintered at different temperatures and corresponding detail of a representative strut of the porous ceramics. The results reveal that the sintering process and densification degree increase with sintering temperature. A microstructure with fully densified polycrystalline matrix and struts is clearly seen for the samples sintered at 1200 °C for 3 h, indicating that the HAp powder is well sintered under...
these conditions. The densification of particles in the continuous phase during drying and at the droplet interface during sintering results in the formation of these dense struts and cell walls. At sintering temperatures above 1200 °C, the pores begin to close and collapse, leading to the porosity dropping below 70%, as shown in Fig. 5. In order to achieve high connectivity, we choose 1200 °C as the optimum sintering temperature. Compared with the conventional polymer template method, it is possible to effectively avoid residual porosity in the struts, which results from the pyrolysis of the polyurethane foam, thus resulting in improved mechanical strength of the structure.

4. Conclusions

An attractive alternative route that uses particle-stabilized emulsions as templates for the preparation of porous HAp ceramics was developed. The high open porosity, mechanical strength, and promising 3D interconnected macropore structure allows for further development of bone-repair materials with excellent osteoconductivity and bioactivity for hard tissue applications. The scaffolds have a homogeneous pore size ranging from 5 to 30 μm. We achieved a compressive strength of 4.26 MPa and bending strength of 1.69 MPa for scaffolds with a porosity of 76.5%.

An industrialized extrusion or tape-casting process can be used to easily fabricate green ceramic supports that benefit from the long-term stability and viscoelastic behavior of the particle-stabilized emulsions. In future work, investigation on osteoconductivity and bioactivity may further demonstrate the potential advantages of these materials in BTE.

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References


