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Fabrication and cellular biocompatibility of porous carbonated biphasic calcium phosphate ceramics with a nanostructure

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Abstract

Microwave heating was applied to fabricate interconnective porous structured bodies by foaming as-synthesized calcium-deficient hydroxyapatite (Ca-deficient HA) precipitate containing H_2O_2. The porous bodies were sintered by a microwave process with activated carbon as the embedding material to prepare nano- and submicron-structured ceramics. By comparison, conventional sintering was used to produce microstructured ceramics. The precursor particles and bulk ceramics were characterized by transmission electron microscopy (TEM), dynamic light scattering, scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier-transformed infrared spectroscopy (FTIR) and mechanical testing. TEM micrographs and assessment of the size distribution showed that the needle-like precursor particles are on the nanoscale. SEM observation indicated that the ceramics formed by microwave sintering presented a structure of interconnective pores, with average grain sizes of ~86 and ~167 nm. XRD patterns and FTIR spectra confirmed the presence of carbonated biphasic calcium phosphate (BCP), and the mechanical tests showed that the ceramics formed by microwave sintering had a compressive strength comparable to that obtained by conventional methods. Rat osteoblasts were cultured on the three kinds of BCP ceramics to evaluate their biocompatibility. Compared with the microscale group formed by conventional sintering, MTT assay and ALP assay showed that nanophase scaffolds promoted cell proliferation and differentiation respectively, and SEM observation showed that the nanoscale group clearly promoted cell adhesion. The results from this study suggest that porous carbonated biphasic calcium phosphate ceramics with a nanostructure promote osteoblast adhesion, proliferation and differentiation. In conclusion, porous carbonated BCP ceramics with a nanostructure are simple and quick to prepare using microwaves and compared with those produced by conventional sintering, may be better bone graft materials.

Keywords: Nanostructure; Porous; Carbonated; Biphasic calcium phosphate; Microwave

1. Introduction

With the development of nanotechnology, bone apatite has been proved to consist of nanosized carbonated calcium phosphate crystals [1]. Many studies have shown that bone-forming cells are accustomed to interacting with nanoscale surfaces of biomaterials, and this nanoscale feature of implanted materials is critical to keep the body from rejecting artificial parts [2,3]. It has gradually been recognized that a nanoscale surface could promote the adhesion, proliferation and differentiation of osteoblasts [4,5]. In addition, the bony apatite contains a significant amount of carbonate ions (3–8 wt.%) [6]. According to the previous studies preparing carbonated hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), the carbonate groups would increase its bioactivities [7,8]. Therefore, porous apatite ceramics mimic bony apatite in chemical and structural composition may be better bone grafts.

Porous BCP ceramics containing HA and tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) phases as an ideal bone graft

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substitute have recently attracted great attention due to the similarity of their chemical composition and porous structure with bone mineral [9]. The conventional foaming method of fabricating an interconnective porous structure is complex and time consuming [10], which limits the application of porous BCP ceramics. On the other hand, the sintering method of porous biphasic calcium phosphate (BCP) ceramics is generally based on a conventional heating process with a long time and a high temperature, which produces a grain size greater than that of bone apatite crystal and reduces the bioactivity [11].

A microwave process has been applied to fabricate ceramics, especially nanosized or sub-microsized ceramics, in past decade [12,13]. This novel method has several advantages over conventional sintering, such as a lower sintering temperature (100 °C lower) and a shorter sintering time (only a few minutes). However, CaP ceramics lack the ability to absorb the microwave power under 800 °C. Therefore, it is necessary to choose a proper embedding material to help to elevate temperature under 800 °C.

Many previous studies have focused on the fabrication of nanosized CaP powder and dense CaP ceramics and the evaluation of their cellular biocompatibility [14,15]. To the best of our knowledge, few studies have prepared porous nanosized BCP ceramics. In this study, we used a wet precipitation method to synthesize nanosized Ca-deficient HA as a precursor. After aging and washing, the as-synthesized precipitate was directly foamed with H_2O_2, assisted by microwave heating. Subsequently, another microwave sintering process with activated carbon as the embedding material was conducted to produce porous carbonated BCP ceramics with nanosized or sub-micron-sized grains. Conventional sintering was used to produce microstructured BCP as control. The current study aimed to prepare the novel bone graft and compare its cellular biocompatibility with that of conventional porous ceramics.

2. Materials and methods

2.1. Preparation of nanosized Ca-deficient HA precursor

The flow chart shown in Fig. 1 outlines the experimental procedure used to process porous BCP ceramics in this study.

Ca-deficient HA was synthesized as the precursor by the wet precipitation method. Analytically pure calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$) and diammonium phosphate ($(NH_4)_2HPO_4$) were dissolved into deionized water with concentrations of 0.5 and 0.3 M, respectively, and the initial Ca/P ratio was 1.5. Next, 3 wt.% citric acid (CA) was added to $Ca(NO_3)_2$ solution as a dispersant, and the pH value was kept at 9.5 with ammonia solution. $(NH_4)_2HPO_4$ solution was dropped into the solution of $Ca(NO_3)_2$ with continuous stirring at 55 °C. After adding $(NH_4)_2HPO_4$, the suspension was stirred for another hour then aged for

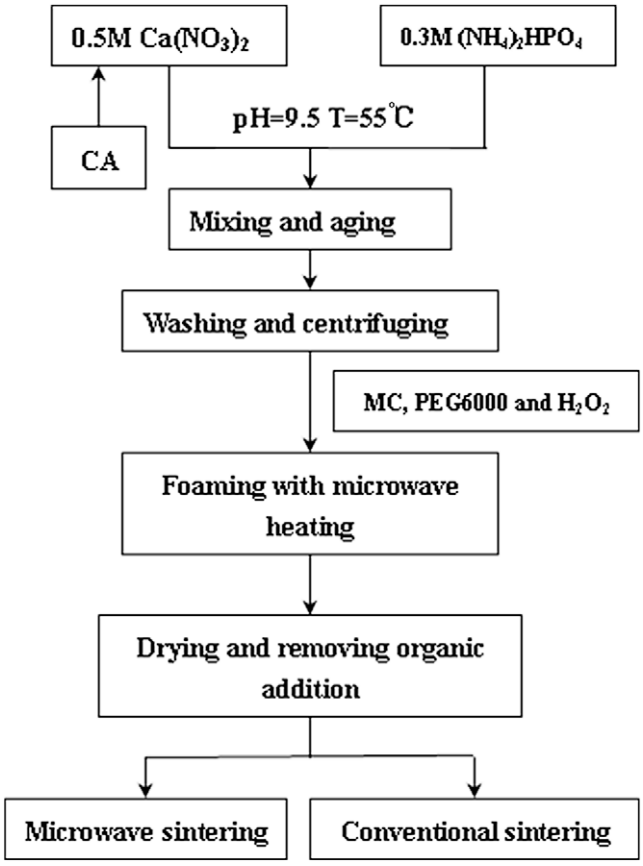

Fig. 1. A flow chart of fabricating porous BCP bioceramics.

36 h at 25 °C. The white precipitation was centrifuged and washed with deionized water for six times. Subsequently, 0.2 wt.% analytically pure methylcellulose (MC) and 0.4 wt.% polyethylene glycol with a molecular weight of 6000 (PEG6000) were added to the precipitate as dispersant and viscous agent. With 6 vol.% analytically pure H_2O_2 as a vesicant, the slurry was heated by microwave (domestic microwave oven, Galanz, China) for 30–60 s and the foaming slurry was poured immediately into the molds with good permeability. After drying at 48 °C for 12 h and removing the organic addition at 550 °C for 6 h, porous green bodies were produced.

2.2. Preparation of porous ceramics by microwave sintering and conventional sintering

The as-prepared porous green bodies were transferred into a microwave furnace for sintering. The set-up of the microwave-sintering furnace is shown in Fig. 2. The microwave sintering furnace was refitted from the domestic microwave oven (2.45 GHz/750 W, Sanyo, Japan). Samples were embedded by activated carbon filled in a hollow porous mullite cube. The temperature of specimens in microwave furnace was measured by an optical fiber pyrometer in the range of 700–1400 °C. The BCP green bodies were sintered by microwave at 950 and 1050 °C for 1 min to obtain nanosized BCP ceramics (NBCP) and

ID	Title	Pages
2353	Fabrication and cellular biocompatibility of porous carbonated biphasic calcium phosphate ceramics with a nanostructure	10