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A simple pathway in preparation of controlled porosity of biphasic calcium phosphate scaffold for dentin regeneration

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Abstract

A simple pathway in preparation of biphasic calcium phosphate scaffold of hydroxyapatite/beta-tricalcium phosphate with controlled pore size, shape and porosity using phosphoric acid and calcium carbonate was successfully developed. Microporosity was controlled by adjusting temperature and soaking time of the sintering process while macroporosity was obtained through addition of polyethylene spherical particles. The advantage of this method is that a highly pure biphasic calcium phosphate scaffold consisting of hydroxyapatite/beta-tricalcium phosphate in a controlled ratio of 20/80 with a mean pore size of 300 μm and 65% porosity can be produced. These properties of scaffold are of high potential for use in dentin regeneration.

Keywords: Biphasic calcium phosphate; Hydroxyapatite/Beta-tricalcium phosphate; Scaffold; Dentin regeneration

1. Introduction

Tissue engineering is a novel concept to regenerate functional new tissue using cells, bioactive molecules and scaffolds. A well-designed scaffold can provide a structural framework as well as a microenvironment to facilitate tissue formation [1,2]. Thus, the selection of appropriate scaffold parameters such as scaffold material, porosity, pore size, shape and pore distribution is critical in dentin tissue regeneration [3,4].

Biphasic calcium phosphate (BCP) materials have been reported as a suitable scaffold for hard tissue engineering [5–8]. BCPs consist of an intimate mixture of hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH)_2$], and beta-tricalcium phosphate (β-TCP) [$Ca_3(PO_4)_2$] crystals of varying phase compositions (HA/β-TCP ratios) [9]. HA is bioactive and β-TCP is resorbable [10], and recent research has been devoted to manipulation of the composition of HA and β-TCP [11,12]. BCP of HA/β-TCP in a ratio of 20/80 has been reported as an appropriate scaffold material for bone tissue engineering and is more effective than pure HA or β-TCP alone [13] and also provided the best dentinogenic effects resulting in a thicker reparative dentin bridge and quicker formation of normal tubular dentin when it was placed in direct contact with pulp tissue [14]. Yıldırım et al. [15] reported that it served as an ideal calcium phosphate biomaterial to induce initial healing response and served as a good carrier for biologically active dentinogenic molecules.

Generally, there are two main methods to synthesize BCP: (1) sintering of calcium-deficient hydroxyapatite (CDHA) powder and (2) direct mechanical mixing of HA and β-TCP powders [16]. In the first method, a synthetic, natural or biological calcium-deficient hydroxyapatite (CDHA) [$Ca_{10-x}(PO_4)_{6-x}(HPO_4)x(OH)_{2-x}$] is sintered at a temperature above 700 °C. The extent of calcium deficiency (Ca/P molar ratio >1.67) determines the HA/β-TCP ratio in the BCP ceramics, which affects the reactivity of the material; the lower the ratio, the higher the reactivity. However, the reactivity also depends on particle

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size, and micro- and macro-porosity of the BCP that are affected by temperature and duration of sintering as well as by other variables of processing [9,17].

The intermediate product of CDHA in BCP processing is usually produced by the precipitation method [16,18]. In one type of precipitation method that employed $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ other phases of impurities resulted [19–21]. $Ca(OH)_2$ and H_3PO_4 were used in another precipitation process. However, the absorption of atmospheric CO_2 by $Ca(OH)_2$ and its partial transformation to $CaCO_3$ resulted in irreproducibility of BCP characteristics [9,22]. Hence, $CaCO_3$ was used due to its chemical stability during storage at standard conditions. The following reaction occurs between $CaCO_3$ and H_3PO_4 [9]:

$$(5-2x)CaCO_3+(3-x)H_3PO_4 \rightarrow (1-x)Ca_5(PO_4)_3 OH + xCa_3(PO_4)_2 + (4-x)H_2O + (5-2x)CO_2 \quad (1)$$

$$x = 10-6Ca/P; \quad 0 \le x \le 1$$

In the case of direct mechanical mixing, the impurities in the initial commercial powders of HA and β-TCP affected the scaffold functional characteristics [16,17]. Another important factor that can also affect the functional characteristics of the scaffold is the porosity, in which pore size and total porous volume are very important. Microporosity (diameter $< 10\,\mu m$) allows body fluid circulation, whereas macroporosity (diameter $> 100\,\mu m$) provides a scaffold for cell attachment, migration, colonization and tissue deposition [23,24].

Many methods have been described for the preparation of macroporous scaffolds: use of polymeric sponges [25,26], porogen substances such as naphthalene particles [27], poly vinyl butyral (PVB) [28], polystyrene or polymethyl methacrylate beads [29], sodium chloride grains [30], flour [31] and gas-forming [32]. However, each technique has its own limitations and challenges. One of the serious challenges is controlling the total porosity, macropores shape and size. It was reported [33] that the total porosity of 65% was efficient compared to those of 25% and 75%, for total protein production and alkaline phosphatase (ALP) activity which were taken as indicators of growth/matrix production and for the comparison of cell differentiation. Tonomura et al. [5] concluded that the porous scaffold of 300 μm pore size was adequate for porcine dental pulp-derived cells to align with the scaffold surface, differentiate, and regenerate dentin-like tissue compared to granular and fiber-type scaffolds. Recently, Ando et al. [14] found that the transplanted dental pulp-derived cells using 2:8 ratio of HA/β-TCP of 300 μm pore size as a scaffold, regenerated dentin bridge-like structures on exposed dental pulp tissue. The hard tissue was positive for type I collagen (Col-1), osteonectin (ON), bone sialoprotein (BSP), and dentin sialoprotein (DSP), which are important markers for dentin.

The preparation of pure BCP scaffold with HA/β-TCP ratio of 20/80, controlled total porosity and macropores suitable for dentin regeneration has not been reported in the literature and hence the aim of this study was to develop a simple pathway for preparation of this material. The shape and size of the macropores were controlled by using a porogen, polyethylene (PE) spherical particles. Thermogravimetry analysis (TGA) and differential thermal analysis (DTA) were used to adjust thermal treatment during scaffold fabrication. Qualitative and quantitative X-ray diffraction (XRD), X-ray fluorescence spectroscopy (XRF) and fourier transform infrared (FTIR) were used for scaffold characterization. Pore size, shape and distribution were evaluated using a field emission scanning electron microscope (FESEM) and image analyzer. The total porosity was measured by the Archimedes method.

2. Materials and methods

2.1. BCP scaffold synthesis

BCP was processed by CDHA synthesis using the wet precipitation method. To prepare BCP of 20/80 ratio of HA/β-TCP, different Ca/P ratios were used. For whole ratios, H_3PO_4 (99.99%, Aldrich, USA) water solution was added at a rate of 7 mL/min into a water suspension of $CaCO_3$ ($\geq$ 99.0%, Aldrich, USA) under continuous stirring at room temperature (28 ± 3 °C). When the pH of the medium was 7.0 or 8.0, the mixture was heated at a temperature of 80 °C for an hour with stirring before cooling it down to room temperature. The precipitate was aged for 48 h, then washed three times and filtered using a centrifuge and dried using an oven at 100 °C for 24 h. The dried cake was crushed using pestle and mortar and the powder of mean particle size of 0.97 μm was mixed with PE spherical particles of 300–350 μm (Cosphere, USA) at a ratio of 4:2.5 v/v using alumina balls. The mixture was uniaxially pressed at 24 MPa in a 32 mm die to form 6 mm thick pellets.

To optimize the heating profile and to avoid cracks that form due to expansion of beads when heated in air, TGA–DTA analyses were performed on a mixture of CDHA/PE. The analysis was performed at a heating rate of 10 °C/min from 26 °C to 1000 °C. On the basis of TGA/DTA study, the pellets were fired at 400 °C for 2 h with 0.5 °C/min heating rate for PE removal and further sintered at 1000 °C for 2 h with 5 °C/min before cooling down to room temperature. Scaffold synthesis procedure is schematically illustrated in Fig. 1.

2.2. Characterization of the scaffold

The phase present and the ratio of HA/β-TCP of the scaffold were analyzed qualitatively and quantitatively by the XRD analysis using Eva and X'Pert HighScore Plus softwares, respectively. The scanning was done from 10° to 60° at a rate of 0.02°/min (Bruker, D2, PHASER) using Cu-Kα radiation generated at 30 kV and 10 mA. The crystalline phases were determined from a comparison of