Materials Science & Engineering C 82 (2018) 217–224

Contents lists available at ScienceDirect

Materials Science & Engineering C

journal homepage: www.elsevier.com/locate/msec

Hierarchically porous structure, mechanical strength and cell biological behaviors of calcium phosphate composite scaffolds prepared by combination of extrusion and porogen burnout technique and enhanced by gelatin

Shenglei Feng[a,b], Fupo He[c,*], Jiandong Ye[a,b,d,**]

[a] School of Materials Science and Engineering, South China University of Technology, Guangzhou 510641, China
[b] National Engineering Research Center for Tissue Restoration and Reconstruction, Guangzhou 510006, China
[c] School of Electromechanical Engineering, Guangdong University of Technology, Guangzhou 510006, China
[d] Key Laboratory of Biomedical Materials of Ministry of Education, South China University of Technology, Guangzhou 510641, China

ARTICLE INFO

Keywords:
Calcium phosphate
Gelatin
Scaffold
Extrusion
Compressive strength

ABSTRACT

In this study, hierarchically porous calcium phosphate scaffolds (HTCP) with unidirectional pores, transversely interconnected pores, and micropores were fabricated by the combination of extrusion and porogen burnout technique. Gelatin was incorporated into the HTCP scaffolds by vacuum-impregnation of gelatin solution and subsequent freeze-drying. The phase composition, microstructure, physical and cytobiological properties were analyzed. The results showed that the HTCP scaffolds were composed of β-tricalcium phosphate with minor hydroxyapatite. The HTCP scaffolds had unidirectional pores (~400 μm), transversely interconnected pores (~130 μm) and micropores (~1 μm). The incorporation of gelatin significantly increased the compressive strength, toughness, and cell seeding of the HTCP scaffolds. The composite scaffolds showed excellent cytocompatibility. The hierarchically porous calcium phosphate composite scaffolds may have potential application prospects in bone tissue engineering.

1. Introduction

Tissue engineering approach, which employs cells, scaffold and growth factors, is emerging as a promising alternative to reconstruct impaired tissues [1]. As an essential component of bone tissue engineering, porous scaffold provides a temporary structural support for cell seeding, growth of cells, and new bone tissues. Ideal scaffold should be biocompatible, biodegradable and osteoconductive, and possess appropriate pore structure, including high porosity, proper pore size (200–600 μm), and high three dimensional (3D) interconnectivity [2]. In addition, the presence of micropores is beneficial for bone formation [3]. To date, a number of biomaterials have been developed, including polymer, ceramic, metal, and their hybrids [4–15]. Among them, calcium phosphates, including hydroxyapatite (HA), β-tricalcium phosphate (TCP), and their mixtures (HA/TCP), are widely used because they possess excellent biocompatibility and osteoconductivity [16–17]. HA degrades slowly in physiological environment, which inhibits the replacement process by new bone tissues [18]. In comparison, β-TCP is more degradable, and its degradation products (Ca^{2+} and PO_4^{3-}) can take part in bone reconstruction [19].

Porous scaffolds can be prepared by a variety of methods, such as polymer templating [20], porogen burnout [21], freeze drying [22], foaming [23], and 3D printing [24]. Extrusion technique has been widely used for manufacturing honeycomb ceramics, which are characterized by high porosity, channel-like pores and controllable pore structure. It is well-known that honeycomb ceramics are commonly used as catalyst support, regenerator and filter [25]. In our previous study, we first prepared honeycomb β-TCP scaffold employing extrusion technique [26]. However, honeycomb β-TCP bioceramic scaffold lacks 3D pore interconnectivity, which is a critical factor for nutrients transport, waste outflow, and vascularization.

Natural bone is a porous composite made up of organic and mineral phases. Porous bioceramics feature intrinsic brittleness and weak mechanical strength, so their wide application for bone repair is restricted. By incorporating polymer, the drawbacks of bioceramics can be resolved to some extent. Up to now, a variety of biopolymer/bioceramic

* Corresponding author.
** Correspondence to: J. Ye, National Engineering Research Center for Tissue Restoration and Reconstruction, Guangzhou 510006, China.
E-mail addresses: fphe@gdut.edu.cn (F. He), jdye@scut.edu.cn (J. Ye).

http://dx.doi.org/10.1016/j.msec.2017.08.039
Received 10 May 2017; Received in revised form 26 July 2017; Accepted 10 August 2017
Available online 12 August 2017

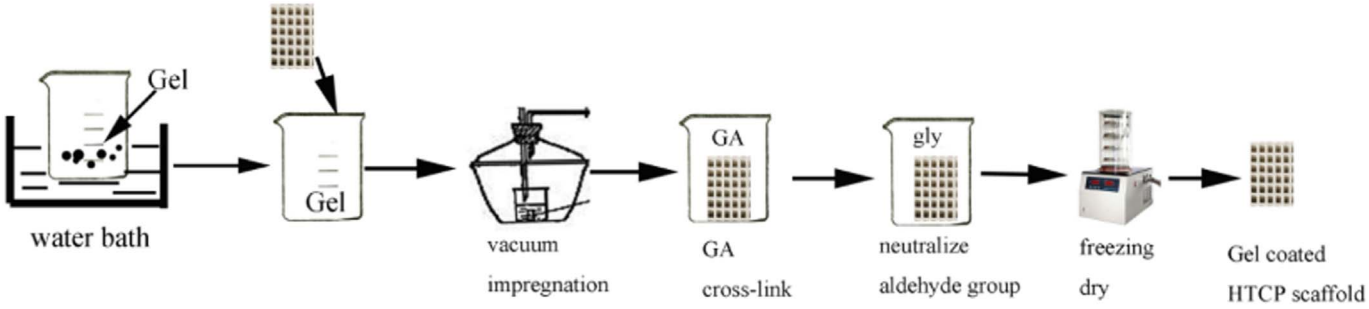

Fig. 1. Schematic diagram of fabricating gelatin-incorporated HTCP scaffolds.

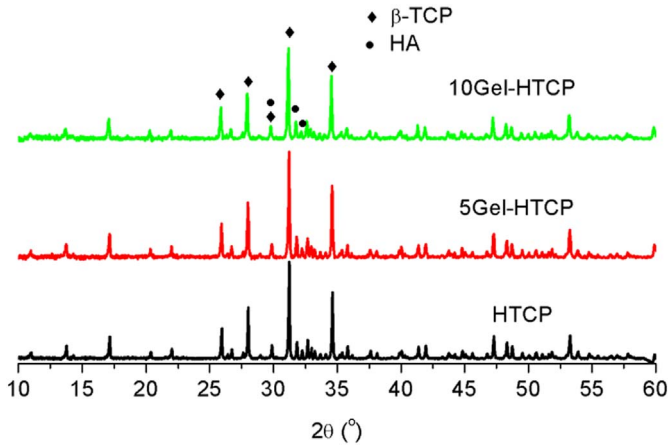

Fig. 2. XRD patterns of HTCP, 5Gel-HTCP and 10Gel-HTCP scaffolds.

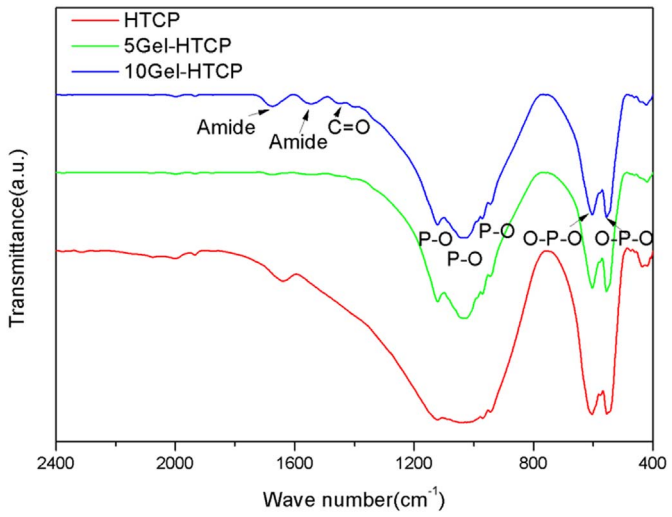

Fig. 3. FTIR spectra of HTCP, 5Gel-HTCP and 10Gel-HTCP scaffolds.

composite have been developed, such as HAp/collagen [27], HA/PA66/chitosan [28], HA/gelatin/poly(lactic acid) [29], TCP/collagen [30], and TCP/poly(lactic-*co*-glycolic acid) [31]. Collagen is the main organic constituent of natural bone. As a natural biopolymer and hydrolyzed form of collagen, gelatin is able to enhance osteoblast adhesion, migration and mineralization, due to its biological functional groups, such as amino acids within the backbone [32].

Considering the lack of 3D interconnectivity of honeycomb bioceramics, in this study, we aimed to fabricate hierarchically porous calcium phosphate scaffolds (HTCP) with unidirectional macropores, transversely interconnected pores and micropores by the combination of extrusion and porogen burnout technique. To overcome the intrinsic poor mechanical property of porous ceramics, gelatin was incorporated into porous β-TCP scaffolds by impregnation of gelatin solution, followed by freeze-drying. The phase composition, microstructure, physical and cytobiological properties of the scaffolds were systematically evaluated.

2. Materials and methods

2.1. Fabrication of scaffolds

β-TCP powder was synthesized by a solid-phase reaction method [33]. Gelatin microspheres (GM) with sizes ranging from 212 to 250 μm were prepared employing a microemulsion method [34]. The HTCP scaffolds were fabricated by the combination of extrusion and porogen burnout technique. GM and polymethyl methacrylate (PMMA) microspheres (0.4–20 μm, SOKEN, Japan) were used as transversely interconnected pore-forming agent and micropore-forming agent, respectively. Methyl cellulose (MC, Sinopharm, China) was used as a binder. β-TCP powder, GM, PMMA microspheres, and MC were mixed uniformly with a mixer (HR1848, Philips). The mass ratio of β-TCP, GM, PMMA microspheres, and MC in the mixture was 79:8:5:8. Deionized water was added to the mixtures with a solid to liquid ratio of 2.7 g/mL, then manually mixed and kneaded, and a plastic pug was obtained. The pug was placed into an extrusion mold, and then extruded with a speed of 8 mm/min on a multi-functional mechanical testing machine (YAW-3000A, Jinan Tianchen Testing Machine Manufacturing Co., Ltd., China). The obtained green bodies were dried at 60 °C overnight, and finally sintered at 1200 °C for 2 h.

Gelatin was incorporated into the HTCP scaffolds by a vacuum-impregnation of gelatin solution and subsequent freeze-drying. The whole process is shown in Fig. 1. Firstly, gelatin particles were dissolved in deionized water at 60 °C, obtaining gelatin solution (5 wt% or 10 wt%). Then, 1.5 g of the HTCP scaffolds were put into 20 mL of gelatin solution, then subjected to vacuum-impregnation for 1 h. The gelatin impregnated HTCP scaffolds were put into 20 mL 2.5 vol% glutaraldehyde (Kermel Co., China) solution to cross-link gelatin polymeric chains. To remove residual glutaraldehyde, the scaffolds were immersed into 0.1 M glycine (Shanghai Bio Science & Technology Co., China) solution for 1 h. After rinsed three times with deionized water, the scaffolds were lyophilized, and finally the HTCP scaffolds incorporated with gelatin were obtained. The gelatin-incorporated HTCP scaffolds (Gel-HTCP) treated by 5 wt% and 10 wt% gelatin solution were designated as 5Gel-HTCP and 10Gel-HTCP, respectively.

2.2. Scaffolds characterization

The phase composition of scaffolds was analyzed by an X-ray diffractometer (X'Pert PRO, PANalytical Co., the Netherlands), employing CuK_α radiation (1.54 Å). The instrument voltage and current were set as 40 kV and 40 mA, respectively. The 2θ range was from 10° to 60° with 0.013° step size and 10 s step time. The functional groups were determined by Fourier Transform Infra-Red Spectroscopy (FTIR, Thermo