Materials Letters 128 (2014) 179–182

Contents lists available at ScienceDirect

Materials Letters

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

Fabrication and characterization of porous 3D whisker-covered calcium phosphate scaffolds

Xingjiang Ye, Changchun Zhou *, Zhanwen Xiao, Yujiang Fan, Xiangdong Zhu, Yong Sun, Xingdong Zhang *

National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, China

ARTICLE INFO

Article history:
Received 23 January 2014
Accepted 22 April 2014
Available online 30 April 2014

Keywords:
Calcium phosphate
Hydrothermal treatment
Porous structures
3D whiskers
Bone tissue engineering

ABSTRACT

Porous three-dimensional (3D) whisker-covered calcium phosphate (CaP) scaffolds were fabricated using a hydrothermal method. Porous CaP ceramics were first prepared by applying the H_2O_2 foaming method to mimic the porous structure of bones. Then, three-dimensional (3D) hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) micro-whiskers were generated in CaP ceramics by hydrothermal treatment. Scanning electronic microscopy (SEM) images revealed that the scaffolds maintained their porous structures and newly generated micro-whiskers with 3D morphologies were vertically grown on ceramic inner walls. Energy dispersive X-ray (EDX) and X-ray diffraction (XRD) confirmed that the micro-whiskers are made of HAp microcrystals. The HAp whisker formation mechanism was proposed. 3D HAp whisker-covered porous CaP ceramics provide a structural design reference for bone tissue engineering.

1. Introduction

Calcium phosphate (CaP) is extensively used in bone tissue engineering as a scaffold because of its similarity to the components of natural bone and excellent biocompatibility, bioactivity, osteoconductivity, and osteoinductivity [1]. For bone tissue applications, scaffolds must have the appropriate three-dimensional (3D) geometry to promote cell attachment, proliferation, differentiation, and metabolite transportation [2,3]. However, the mechanical properties of scaffolds sharply decrease as their porosity increases. Therefore, the development of porous 3D CaP scaffolds with better mechanical strength is of great importance [4].

CaP scaffolds with enhanced mechanical strength may be obtained after sintering at high temperatures [5] but these scaffolds are achieved at the expense of poorer bioactivities. Other common approaches to improve the mechanical properties involve incorporation of reinforcing phases in the form of particles, fibers, and whiskers [6–8]. Carbon particles [9], organic fibers [10], carbon nanotubes [11], and diopside whiskers [12] have been prepared for such purposes. However, whether or not these reinforcements are as biocompatible as pure CaP materials is unknown. Introduction of foreign materials into a CaP matrix may lead to a decrease in biocompatibility and impairment of CaP scaffold biodegradability. Several researchers have reported that hydrothermally synthesized hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) fine crystals/whiskers can be used as reinforcement materials [13,14]. Porous materials with improved strength may be fabricated using HAp fibers or whiskers. Porous materials are reported to exhibit improved strength due to interlocking of fibers, reorganization/filling of micro gaps, crack deflection, and/or pull-out [15]. From this point of view, biomaterials reinforced with HAp whiskers appear to be an excellent option because they are biocompatible reinforcement. Besides, the surface topographical microstructures of a biomaterial greatly affect the biological behavior of cells. Improvement of the specific surface area of biomaterials is believed to result in stronger interactions with living organisms and improved biological activity [16].

In this study, a hydrothermal method is used to prepare 3D HAp whiskers in porous CaP scaffolds. A porous CaP ceramic was initially designed to mimic the structure of porous bone, provide a sufficient calcium source, and achieve the desired mass transport properties. Then, the hydrothermal method is applied to induce 3D HAp whisker growth in the CaP ceramic. The morphologies and formation mechanism of HAp whiskers were studied. Results indicate that porous CaP scaffolds with a controlled morphology of 3D HAp whiskers can be obtained by using the hydrothermal method.

2. Materials and methods

Preparation of porous 3D whisker-reinforced calcium phosphate scaffolds: Biphase calcium phosphate (BCP) powder (hydroxyapatite/β-tricalcium phosphate=20:80) was synthesized using a wet chemical

* Corresponding authors. Tel.: +86 28 85412757; fax: +86 28 85410246.
E-mail addresses: changchunzhou@scu.edu.cn (C. Zhou), zhangxd@scu.edu.cn (X. Zhang).

http://dx.doi.org/10.1016/j.matlet.2014.04.142
0167-577X/

method in our laboratory. Five liters of 0.85 mol/L ($Ca(NO_3)_2$) solution was dropped slowly into 5 L of 0.55 mol/L $(NH_4)_2HPO_4$ solution under stirring at room temperature. The pH of this solution was maintained at 8 by addition of ammonium hydroxide solution. The reaction solution was stirred for 4 h and aged for 24 h. The reaction precipitate was then collected, washed with deionized water, dried, and crushed into powder with a particle size of about 200 μm [17]. The porous CaP green body was designed using the H_2O_2 foaming method and then sintered at 1200 °C to form ceramics. Samples were cut into Φ5 mm × 10 mm cylinders using an outer edge diamond cutter with a speed of 1500 r/min. In the second stage of scaffold fabrication, the hydrothermal method was conducted to generate micro-whiskers. Exactly 1 mol/L nitric acid was mixed with deionized water to prepare the reactor solution at pH=4.0–5.0. CaP ceramics were immersed in 70 mL of the reaction solution and sealed in a 100 mL Teflon reactor. The Teflon reactor was heated to 180 °C at a speed of 10 °C/min. Different dwell times were employed to generate whiskers of different morphologies. The dwell time, which directly affects the length and diameter of the whiskers was set to 2, 4, 8, and 12 h. Fig. 1 shows a schematic diagram of the preparation of porous 3D whisker-reinforced calcium phosphate scaffolds.

Characterization: Scanning electron microscopy (SEM; JSE-5900LV, Japan) was used to observe the scaffold microstructures. Local elemental analysis was carried out by energy-dispersive X-ray spectroscopy (EDX; Oxford, IE250, UK) to assist with phase identification. The crystalline phase was analyzed using X-ray diffractometry (XRD; Philips X'Pert 1 X-ray diffractometer, Netherlands) with CuKα radiation at a current of 20 mA and voltage of 30 kV. Scans were performed with 2θ values from 20° to 60° at a rate of 0.05° s^{-1}. The obtained peaks were compared with standard references for HAp (09-0432) and β-TCP (09-0169) in the JCPDS file available in the software [18].

3. Results and discussion

The porous scaffold and its microstructures are shown in Fig. 2 (a) and (b) (before HAp whisker formation) and Fig. 2(c) (after HAp whisker formation), respectively. The porosity of the sintered CaP scaffolds was measured by the mercury intrusion method. The average porosity of the bulk ceramics was 75% ± 10% and the pore size was 270 ± 80 μm (Fig. 2b). The image-processing software Image-pro Plus was used for crystal and whisker size measurements. The high-magnification image shown in Fig. 2(b) indicates that the ceramic surface is composed of smooth calcium phosphate grains with a diameter of 2.2 ± 0.9 μm. However, after hydrothermal treatment, a layer of three-dimensional micro-whiskers was observed in the porous CaP scaffolds (Fig. 2(c)–(i)). These micro-whiskers grew vertically on the inner pore walls of the scaffolds and featured diameters ranging from 200 nm to 1 μm, as calculated from the SEM images.

Fig. 3 shows the process of HAp whisker formation. Fig. 3 (a) shows the initial morphology of the CaP scaffold surface. Fig. 3 (b) shows the status of newly formed calcium phosphate nuclei at the first instance of hydrothermal treatment. Fig. 3(c) and (d) shows that the whiskers gradually grow from nucleus-like dots into several micrometer-length whiskers. Fig. 3(e) and (f) shows the final morphology of the HAp whiskers. After full crystallization, the HAp whiskers eventually form hexagonal rods. Fig. 3(f) shows a magnified image of the HAp whiskers.

We studied the effect of CaP phase composition on HAp whisker formation. While BCP (containing β-TCP and HAp) and β-TCP ceramics easily formed HAp whiskers, formation of HAp whiskers on a HAp ceramic matrix was difficult. Based on experimental results, we speculate that the formation of HAp whiskers follows a dissolution–redeposition mechanism. β-TCP showed the best solubility among different phases of calcium phosphate ceramics; by contrast, HAp was dissolved with difficulty. Therefore, the formation of HAp whiskers is believed to be due to the dissolved calcium and phosphate ions from β-TCP. During hydrothermal treatment, the β-TCP in the ceramic matrix quickly dissolves into calcium and phosphate ions because of its high solubility. These ions are then redeposited into the ceramic surface and become crystals once the ion concentration reaches the recrystallization threshold. Thus, HAp whisker formation may be explained via a two-steps dissolution–redeposition reaction [19]:

$$Ca_3(PO_4)_2 \cdot H_2O \rightarrow Ca^{2+} + 2PO_4^{3-} + H_2O \quad (1)$$

$$10Ca^{2+} + 6PO_4^{3-} + H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 \quad (2)$$

The morphology of the HAp whiskers can be controlled by the hydrothermal treatment time. To determine the average whisker length, we randomly selected 20 whiskers from the SEM images. As shown in Fig. 4, whiskers with a length of 2.32 ± 0.23 μm (statistical data) and an aspect ratio of 4.6 were formed on the porous CaP matrix after 0.5 h of hydrothermal treatment. When the hydrothermal treatment times were prolonged to 1, 2, 4, and 8 h, the whiskers grew to mean lengths of 7.75 ± 0.40 μm, 37.39 ± 2.86 μm, 53.21 ± 2.83 μm, and 95.96 ± 9.83 μm, respectively. The aspect ratio of the whiskers showed a similar growth trend, reaching 17.0, 39.1, 62.5, and 81.5 after treatment for 1, 2, 4, and 8 h, respectively. Once the HAp whiskers reached full crystallization, growth ceased. The length of the whiskers decreased to 56.21 ± 3.99 μm and their aspect ratio decreased to 78.5 when the hydrothermal treatment time was extended to 12 h.

The micro-whiskers were analyzed using EDX and XRD. Elemental micro-analysis of six tested specimens yielded an average Ca/P ratio of 1.71 ± 0.43, which is close to the stoichiometric HAp of 1.67. The typical XRD pattern of the whiskers is shown in Fig. 5.

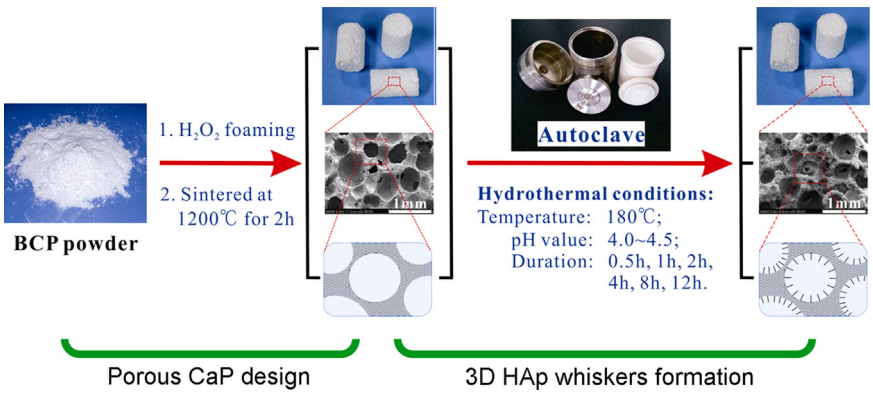

Fig. 1. Preparation of porous 3D whisker-reinforced calcium phosphate scaffolds.