FABRICATION AND EVALUATION OF SILICON-CONTAINING APATITE FIBER SCAFFOLDS FOR BONE TISSUE ENGINEERING

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Abstract: The aim of this work is to develop a highly-functional tissue engineering scaffold from silicon-containing apatite fibres (Si-AF). Firstly, Si-AFs were synthesized by a homogeneous precipitation method. Starting solution with a Ca/(P+Si) ratio of 1.67 was prepared by mixing $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{HPO}_4$, $\text{Si}(\text{OC}_2\text{H}_5)_4$ (TEOS), $(\text{NH}_2)_2\text{CO}$ and HNO_3 . The concentrations of TEOS in the starting solution were 0 (AF), 0.8 (0.8Si-AF) and 1.6 (1.6Si-AF) mass%. Next, Si-AF scaffolds (Si-AFS) were fabricated by firing the compacts consisting of Si-AF and carbon beads of 150 μm diameter. The resulting Si-AFSs were characterized using X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and inductively coupled plasma atomic emission spectrometry (ICP-AES). Results showed that the material properties of the 0.8Si-AFS were similar to those of the conventional AFS without silicon. Consequently, we can conclude that the 0.8Si-AFS offers as a potential novel scaffold material, creating a three-dimensional cell culture environment.

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INTRODUCTION

Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$; HAp) is one of the main inorganic components found in the biological hard tissue, and it has the ability to bond directly with host tissue. Thus, HAp is considered as a material which is suitable for use in the biomedical applications, such as bone grafts and scaffolds for bone tissue engineering [1-3]. Although the composition of HAp is similar to that of the bone mineral, there are a number of distinct differences between the two materials, in terms of their trace ion contents.

In recent investigations, the bioactivity of HAp has shown to increase via substitution of suitable ions into its crystal lattice. It is known that the silicon (Si) is an element which promotes bone formation [4]. Therefore, Si-substituted HAp (Si-HAp) ceramics have a higher bioactivity as compared to pure HAp ceramics [5]. For example, substitution of a minute amount of Si into the HAp lattice has shown to improve dramatically the rate of bone-bonding ability with the implant materials [6].

The authors have successfully synthesized HAp fibre (AF) by a homogenous precipitation method [7]. Using these AFs, three-dimensional apatite-fiber scaffold (AFS) were fabricated which are suitable for cell culture environment [2]. Furthermore, we have

successfully synthesized silicon-containing apatite fibers (Si-AF) by homogeneous precipitation method [8].

Our aim is now to fabricate a highly-functional tissue engineering scaffold from these Si-AFs, followed by evaluating its material properties.

MATERIALS AND METHODS

Synthesis of Si-AFs

The nominal composition of silicon substitution can be described as $\text{Ca}_{10}(\text{PO}_4)_{6-x}(\text{SiO}_4)_x(\text{OH})_{2-x}$. As previously reported in [8], the Si-AFs were synthesized by a homogeneous precipitation method. Starting solution with a Ca/(P+Si) ratio of 1.67 was prepared by mixing $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{HPO}_4$, $\text{Si}(\text{OC}_2\text{H}_5)_4$ (TEOS), $(\text{NH}_2)_2\text{CO}$ and HNO_3 . The concentrations of TEOS in the starting solution were 0 (AF), 0.8 (0.8Si-AF) and 1.6 mass% (1.6Si-AF). The precursor phase for the Si-AF, octacalcium phosphate ($\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$; OCP) fibers, were firstly prepared by heating the starting solution at 80 °C for 24 h. The resulting OCP fibers were then transformed into Si-AF by heating at 95 °C for 144 h in the presence of TEOS. On the other hand, AF (Si content 0 mass%) was prepared by heating at 80 °C for 24 h, followed by heating at 90 °C for 72 h [8].

Fabrication of Si-AFS

Spherical carbon beads with a diameter of ~150 μm were added to the Si-AF slurry (1 mass% Si-AF in the mixed solvent of ethanol/water = 1/1 (v/v)) with a carbon/Si-AF (w/w) ratio of 0, 10/1 and 20/1. The compacts for scaffolds were then fabricated by pouring and vacuum pumping the carbon beads-containing Si-AF slurry into the vinyl-chloride mold. The resulting compacts were fired at 1300 $^{\circ}\text{C}$ for 5 h in a humidified atmosphere to fabricate the Si-AFS. Here, scaffolds with a carbon/Si-AF (w/w) ratio of 0, 10/1 and 20/1 are referred to as Si-AFS0, Si-AFS1000 and Si-AFS2000.

The Si-AFs and Si-AFSs were characterized by X-ray diffractometry (XRD), Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and inductively coupled plasma atomic emission spectrometry (ICP-AES).

RESULTS AND DISCUSSION

Characterization of Si-AFs

Figure 1 shows the typical XRD patterns of the Si-AFs. XRD patterns of the AF, 0.8Si-AF and 1.6Si-AF indicated the single-phase HAp. Figure 2 shows the typical FT-IR spectra of the Si-AFs. In the FT-IR spectra of the above Si-AFs, the absorptions assigned to the PO_4^{3-} groups at 1300-900, 600 and 570 cm^{-1} and assigned to the OH^- group at 3570 cm^{-1} , were detected. In addition, the absorptions assigned to CO_3^{2-} groups were detected at 1460, 1420 and 880 cm^{-1} . These absorptions indicate that the present Si-AFs were consisted of carbonate-containing apatite. The Si content of the Si-AFs increased in proportion to the amount of Si added as given Table 1. Figure 3(a) shows the typical SEM micrograph of the Si-AF. The SEM micrographs indicate that the Si-AFs were approximately 60-100 μm in length.

Fig.1 XRD pattern of the Si-AFs:
(a) 1.6Si-AF, (b) 0.8Si-AF, (c) AF

Fig.2 FT-IR spectra of the Si-AFs:
(a) 1.6Si-AF, (b) 0.8Si-AF, (c) AF

Table 1 Chemical compositions of Si-AFs and Si-AFSs

Sample	Ca / mass%	P / mass%	Si / mass%	Ca/P	Ca/(P+Si)
nominal composition	AF	39.90	18.51	—	1.67
	0.8Si-AF	40.12	17.74	0.80	1.75
	1.6Si-AF	40.35	16.96	1.60	1.67
as-prepared	AF	37.21	16.88	—	1.70
	0.8Si-AF	40.28	18.29	0.66	1.70
	1.6Si-AF	35.56	16.23	1.18	1.69
fired	AFS	39.68	18.32	—	1.67
	0.8Si-AFS	37.88	17.59	1.00	1.66
	1.6Si-AFS	38.61	17.70	1.66	1.53

Fig. 3 SEM micrographs of the Si-AF and the Si-AFSs:
 (a) 0.8Si-AF, (b) 0.8Si-AFS0,
 (c) 0.8Si-AFS1000, (d) 0.8Si-AFS2000.

Characterization of Si-AFSs

Figure 4 shows the typical XRD patterns of the Si-AFSs. The XRD pattern indicated that the AFS was single-phase HAp. However, a small amount of α -tricalcium phosphate (α -TCP) was detected for the 0.8Si-AFS. As for the 1.6Si-AFS, they were thermally decomposed into α -TCP and β -TCP phases. Generally, Si-AFSs decomposed with increasing silicon addition.

Fig. 4 XRD pattern of the Si-AFSs:
 (a) 1.6Si-AFS1000, (b) 0.8Si-AFS1000, (c) AFS1000

Figure 5 shows the typical FT-IR spectra of the Si-AFSs. In the FT-IR spectra, the absorptions assigned to the PO_4^{3-} groups were detected at 1300-900, 600 and 570 cm^{-1} , together with those assigned to the OH^- groups at 3570 cm^{-1} . The CO_3^{2-} groups were not detected after firing.

Fig. 5 FT-IR spectra of the Si-AFSs:
 (a) 1.6Si-AFS1000, (b) 0.8Si-AFS1000, (c) AFS1000

Figures 3(b)-(d) show typical SEM micrographs of the Si-AFSs. The microstructures of 0.8Si-AFS1000 (Fig. 3(c)) and 0.8Si-AFS2000 (Fig. 3(d)) contained lots of macropores of sizes ranging from 100 to 150 μm . The porosity level of 0.8Si-AFS0, 0.8Si-AFS1000 and 0.8Si-AFS 2000 were 90.3%, 97.8% and 98.4%, respectively. It was noted from this work that the porosity level of Si-AFSs increased with the numbers of carbon beads used.

The Ca, P and Si contents of the samples were determined using ICP-AES. Table 1 shows the chemical compositions of Si-AFSs. It revealed that Si was present in the Si-AFSs after firing. In addition, Si composition of Si-AFS was similar to the nominal composition.

The effect of Si substitution on the crystal structure of HAp was examined by determining the lattice constants. The 1.6Si-AFS was not measured due to the presence of TCP phase in the material.

The 0.8Si-AFS resulted in a decrease in the *a*-axis and an increase in the *c*-axis of the unit cell of HAp. This variation of lattice constant was in good agreement with the Si-substituted HAp studies reported by Gibson et al. [5].

It can be deduced that Si is incorporated in the HAp crystal structure from the lattice constant variation of 0.8Si-AFS.

CONCLUSIONS

The Si-AFs were synthesised successfully by a homogenous precipitation method. XRD analysis revealed that these fibres were mainly crystalline HAp phase. Furthermore, the material properties of the 0.8Si-AFS were similar to those of AFS. On the whole, the 0.8Si-AFS offers as a potential novel scaffold material, creating a three-dimensional cell culture environment.

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