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Short communication

Fabrication and characterization of biomorphic 45S5 bioglass scaffold from sugarcane

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ABSTRACT

A biomorphic 45S5 bioglass scaffold has been fabricated from natural plant sugarcane successfully by a novel biotemplating process. Scanning electron microscopy (SEM), X-ray powder diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and thermogravimetry and differential scanning calorimetry (TG-DSC) technologies were employed to characterize the morphology, phase and chemical composition of the products. Experimental results show that the as-fabricated 45S5 bioglass scaffold retained the microstructure of sugarcane very well, and consisted of major crystal phase $Na_2Ca_2Si_3O_9$ of hexagonal system, secondary crystal phase orthorhombic $NaCaPO_4$ and amorphous glass. The biomorphic 45S5 bioglass scaffold may be a promising candidate scaffold for bone tissue engineering.

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1. Introduction

In recent years, a novel replication method, i.e. biotemplating technology using natural plants as sacrificial templates, has been applied to manufacture biomorphic porous ceramics via substitution or transformation processing [1,2].Wood, a typical representative of such biotemplates, exhibits a hierarchical microcellular architecture featuring honeycomb-like microchannels, and has been converted into various ceramics such as carbide (SiC, TiC) [3,4], oxide (Al_2O_3, Cr_2O_3) [5,6] and nitride (TiN) [7] because of its benefits, including easy fabrication of complex shapes, sufficient biomechanical properties [8] and intrinsic three-dimensional interconnected porous structure [9].

The merits allow these biomorphic porous materials for biomedical applications like bone implants and scaffolds in bone reconstitution. Bioactive glass-coated biomorphic ceramics from wood have been verified to possess excellent bone-bonding ability, and are promising devices for dental and orthopaedic applications [10,11]. Moreover, in vitro cytotoxicity observation of biomorphic SiC ceramics, using MG-63 human osteoblast-like cells, has also revealed that the biological response of the cells on the ceramics was similar to those on well-known implant materials like Ti6Al4V and bulk bioactive glass [12].

In fact, there are some reports on direct bone ingrowth into charcoal and wood implants in the recent years. For example, bone ingrowth and appositional growth in small prosthesis implants of juniper, pretreated by a boiling procedure, was reported [13]. Moreover, heat-treated birch was directly used as replacement material for the osteochondral bone defects in the knee joint of rabbit [14]. As a result, the natural porous microchannel structure of wood made it serve as a porous scaffold, which allowed on-growth of bone and cartilage differentiation on its surface and in-growth the porous structure of wood. However, the bioactivity of the wood is not ideal, though its special microstructure facilitates the infiltration and growth of cell and tissue into it. If the microstructure can be converted into bioactive materials such as hydroxyapatite and bioglass, they will become promising candidate scaffolds for tissue engineering strategy. In the past decade, special attention was ever paid to another kind of biomorphic materials for bone regeneration, originating from biological tissues and natural materials like cuttlefish, seashell, seastar and red algae [15,16], etc.

A common problem encountered when using scaffolds for tissue engineering is the rapid formation of tissue on the outer edge, leading to the development of a necrotic core due to the limitations of cell penetration, and oxygen and nutrient exchanges [17]. To overcome this issue, the principal strategy is to adopt dynamic culture systems [18]. However, some recent studies have reported that the issue may also be solved by an alternative approach, i.e., the incorporation of macro- and/or micro-channels within scaffolds [19]. Now, the methods to create the channels in scaffolds mainly include rapid prototyping technology [20], fiber templating [17] and freeze-drying [21], etc. Sugarcane, similar to many other natural plants like wood and bamboo, inherently possesses porous microstructure characteristic of numerous aligned rectangular micro-channels along sugarcane stem direction. A novel biomorphic scaffold of inner micro-channels will possibly be obtained, if the special porous microstructure of sugarcane can be converted to bioactive materials via appropriate methods. Among the biomaterials for bone regeneration, Si and Ca containing silicate glass and glass-ceramics have received special

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interest [22–24], because they can induce the formation of bone-like apatite layer on the surface of scaffolds in vitro when exposed to simulated body fluid.

To the best of our knowledge, there is no report on the preparation of biomorphic bioglass from natural plant templates. In the present study, we utilize, for the first time, the combination of the biotemplating method and sol–gel processing to develop a novel biomorphic 45S5 bioglass from natural plant sugarcane, characterize the morphology, phase and chemical composition of sugarcane-derived 45S5 bioglass, and try to establish a novel method for fabricating ideal bioglass scaffolds for bone tissue engineering application.

2. Experimental procedure

2.1. Synthesis of 45S5 bioglass sol

Tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS, AR, Chengdu Kelong Chemical Reagents Plant, Chengdu, China), calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, AR, Chongqing Beipei Fine Chemical Reagents Plant, Chongqing, China), sodium nitrate ($NaNO_3$, AR, Xi'an Chemical Regents Co., Xi'an, China) and triethyl phosphate ($O=P(OC_2H_5)_3$, TEP, CP, Kunshan Kunhua Chemical Plant, Kunshan, China) were used as precursors to synthesize 45S5 bioglass sol. Briefly, TEOS and TEP were mixed with distilled water and 1 M HNO_3 (AR, Xi'an Chemical Regents Co., Xi'an, China) solution and hydrolyzed for 60 min under vigorous stirring using a magnetic stirrer. Then, $Ca(NO_3)_2{\cdot}4H_2O$ and $NaNO_3$ were gradually added into the mixture. After the reactants were stirred for 6 h, the clear liquid was obtained, namely, 45S5 bioglass sol. The sol was kept in a sealed container for 5 d at room temperature to allow to age. The solid content of the sol was 35 wt.%.

2.2. Preparation of biomorphic 45S5 bioglass scaffold

To obtain biomorphic 45S5 Bioglass, sugar in sugarcane was firstly removed by soaking in hot water for 5 d under shaking condition, and hot water was renewed every 12 h. The remove extent of sugar from the sugarcane was determined by measuring the weight of the samples after different times of soaking in hot water. If the weight did not change any longer with soaking time, it was considered that the sugar in sugarcane was removed completely. The as-treated sugarcane was dried at 65 °C for 12 h in a vacuum oven. Then, the dried sugarcane was infiltrated with the 45S5 bioglass sol via a vacuum/pressure process in a self-made equipment, and dried at 120 °C for 12 h, allowing the occurrence of gelation. The 45S5 bioglass amount in sugarcane was controlled by repeating the infiltration procedure, and about 45 wt.% was chosen in this study. Finally, the 45S5 bioglass-contained sugarcane was heat-treated at 550 °C for 2 h with a heating rate of 2 °C/min in order to remove the organic biotemplate, and then was sintered in air at 1030 °C for 1 h to increase the density of microchannel struts.

2.3. Characterization

The morphologies of sugarcane and biomorphic 45S5 bioglass were characterized by scanning electron microscopy (SEM, Hitachi S-2700, Japan). To determine the crystalline phases of samples, powder X-ray diffraction (XRD) experiment was conducted on an X-ray diffractometer (PANalytical X'Pert Pro, the Netherlands) in the 2θ range of 10–80°, employing Cu$K\alpha$ radiation source operated at 40 kV and 40 mA. The chemical compositions of the samples were determined by Fourier transform infrared spectroscopy (FTIR, Shimadzu IR Prestige-21, Japan) with KBr disc in the wave number range of 4000–400 cm^{-1}. The thermal analysis (TG-DSC) of 45S5 bioglass gel was performed using in a SDT Q600 thermal analyzer (TA Instruments, USA) in air with a heating rate of 10 °C/min.

3. Results and discussion

3.1. TG-DSC analysis of 45S5 bioglass gel

Thermogravimetric analysis and differential scanning calorimetry (TG-DSC) are usually used to determine the temperatures at which physicochemical processes occur. The heat-treatment temperatures in the present study were determined by TG-DSC analysis of 45S5 bioglass gel. Fig. 1 shows TG-DSC curves of 45S5 bioglass gel up to 1300 °C. The DSC curve exhibits five endothermic peaks and one exothermic peak. The first endothermic peak (121 °C) corresponds to the release of physisorbed water and little liquor, which ended at 150 °C (about 12.5% weight loss). The second endothermic peak, starting at 230 °C, corresponds to the pyrolysis reaction of free organic species and/or the release of the resulting water from the further condensation of silanol and P–OH groups, leading to a further 15.5% weight loss. The third and fourth endothermic peaks (centered at 585 °C and 640 °C) are due to the departure of nitrate groups that are usually removed during the thermal stabilization process. The elimination of the species at these temperatures is reflected in the weight loss from TG curve. All the nitrates were removed by 650 °C, leading to an apparent weight loss of 19%. TG curve shows that the total weight reduction is 47%.

Fig. 1 also presents the phase changes of the 45S5 bioglass at higher temperatures. The glass transition of the 45S5 bioglass started at approximately 700 °C. The exothermic DSC peak centralized at about 960 °C with an onset of 905 °C corresponds to the crystallization process of the glass, which approximates to the crystallization temperatures (920 °C) of the glass with a similar composition [25]. The crystal phases of 45S5-based glass-ceramic melted at above 1060 °C (the fifth endothermic peak).

3.2. Morphology of biomorphic 45S5 bioglass scaffold

The porous microstructures of sugarcane and it-derived biomorphic 45S5 bioglass are illustrated in Fig. 2. As seen from the SEM images in Fig. 2a and b, the typical microstructure of sugarcane consists of honeycomb-like channels of rectangular shape (about 20 μm × 30 μm) along the longitudinal direction of sugarcane stem, which are highly interconnected by the pits (with an average diameter of ~10 μm) in the tracheid walls. Fig. 2c and d shows that the as-prepared biomorphic 45S5 bioglass precisely retained the morphology and hierarchical microcellular architecture of sugarcane, and the extensive sintering of 45S5 bioglass particles (<5 μm) in channel struts occurred under the sintering condition (1030 °C/1 h). However, the average pore size (about 10 μm × 18 μm) of biomorphic 45S5 bioglass is smaller than that of sugarcane.

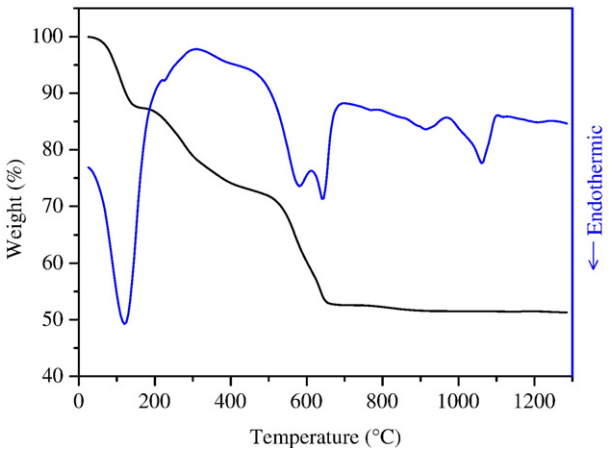

Fig. 1. TG-DSC curves of 45S5 bioglass gel in air.

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