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Fabrication and characterization of silk/forsterite composites for tissue engineering applications

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Abstract

In this research, novel composite scaffolds consisting of silk fibroin and forsterite powder were prepared by a freeze-drying method. In addition, the effects of forsterite powder contents on the structure of the scaffolds were investigated to provide an appropriate composite for bone tissue engineering applications. The morphology studies using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques showed that the forsterite ceramic was well distributed throughout the structures of SF/forsterite scaffolds. Furthermore, the forsterite powder (up to 40 wt%) was homogenously distributed within the silk fibroin as a matrix.

Keywords: Ceramics; Forsterite; Microporous materials; Silk fibroin; Tissue engineering

1. Introduction

Silk fibroin (SF) is a natural polymer produced by a variety of insects. Recently, silk cocoons from *Bombyx mori* have been used as an in-access material for tissue regeneration purposes [1–3]. Silk fibroin exhibits impressive mechanical properties as well as biocompatibility, making it an attractive biomaterial for tissue engineering scaffold preparation. The fibroin protein is a kind of biological material used for the artificial skin and other medical applications. SF is considered as a suitable material for skeletal tissue engineering owing to its good biocompatibility and biodegradability [4,5]. In various applications, the aqueous SF solution has been used for bone regeneration [6–9].

However, a number of problems arise regarding the use of SF in bone tissue engineering applications. The most important problem is the lack of bioactivity of SF.

Additionally, poor mechanical strength of porous SF scaffolds makes them unsuitable for hard tissue engineering. To solve this problem, an important strategy is to combine SF with inorganic materials so that the resulting hybrid materials would possess improved mechanical and biological properties. The optimum range of pore size ceramic scaffolds for bone regeneration was reported to be 80–600 μm [10]. Also, in the design of an ideal scaffold for tissue engineering, compression strength should be between 0.3 and 30 MPa and compressive modulus about 20–1000 MPa. [11–13].

In recent years, some Si–Mg containing bioceramics have been of interest in the development of bone implant materials [14]. Forsterite (Mg_2SiO_4) is a member of olivine family of crystals and is an important silicate (subgroup of nesosilicates) [15–17].

Due to its excellent bioactivity and degradability, forsterite has been proposed as a potential material for the bone tissue regeneration [18]. In addition, nanocrystalline forsterite is a novel bioceramic with high mechanical properties and good biocompatibility, making it suitable for the hard tissue repair even in load-bearing sites [19]. The freeze-drying technique

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has the ability to generate a fine and homogeneous scaffold with excellent control over impurity levels and lower environmental consumption. The current research focused on the synthesis of forsterite/silk fibroin composite scaffolds prepared by the freeze-drying technique.

2. Materials and methods

2.1. Materials

Mg_2SiO_4, $[(MgCO_3)_4 \cdot Mg\ (OH)_2 \cdot 5H_2O]$ and SiO_2 (all supplied by Merck Co., Germany) were used as starting materials. High quality raw cocoons of silkworm, *B. mori*, were purchased from a silk company (Rasht, Iran). To prepare the aqueous silk fibroin solution, distilled water was used. Cellulose dialysis cassettes (Slide-A-lyzer, MWCO 12,000 Da (Sigma)) were used to remove solvent impurities from silk fibroin solution.

2.2. Preparation of regenerated fibroin solution

Silk fibroin (SF) was extracted from silk cocoons according to the protocol previously described with some modifications [14,20]. Briefly, cocoons were boiled several times for 1 h in an aqueous solution of Na_2CO_3 (0.02 M) to remove the glue-like sericin proteins and the remained fibroin was dried. Dry degummed silk fibers were then dissolved in LiBr (9 M) and then the resulting solution was dialyzed against distilled water for three days continuously using a Slide-A-Lyzer dialysis cassette at room temperature to remove the salt. Undissolved particles were removed by centrifugation. The final fibroin solution concentration was about 3.5% (w/w), as determined by weighting the remaining solid of a known volume of solution after drying at 60 °C for two days.

2.3. Forsterite synthesis

The forsterite was prepared according to a procedure described [21]. It was prepared by the conventional solid state ceramic route. Mg_2SiO_4 was used as a basic dielectric material. High purity $[(MgCO_3)_4 \cdot Mg\ (OH)_2 \cdot 5H_2O]$ and SiO_2 were taken as starting materials for the synthesis of forsterite. The chemicals were stoichiometrically weighed and ball milled in a polyethylene bottle in distilled water for 24 h. The final slurry was dried at 100 °C in an oven and then calcined at 1350 °C for 4 h.

2.4. Preparation of forsterite/silk fibroin composite powder

The forsterite/silk fibroin composites were prepared through a freeze-drying method. Initially, certain amounts of forsterite powder were added into the SF solution to obtain three different kinds of samples with ratios of 20:80, 30:70, and 40:60 (forsterite/SF, w/w). This was followed by sonication for 30 min in order to ensure the uniform dispersion of the forsterite powder. The mixture was poured into a pre-cooled 24-well plate, and then put at a temperature of −20 °C for 1 h; after that, it was transferred to a freezer at −80 °C for 12 h and finally freeze-dried in a freeze-dryer for three days. In order to make a comparison, a pure polymer scaffold of freeze-dried SF was prepared without adding any forsterite powders.

2.5. Evaluation of forsterite/silk fibroin composite scaffold properties

The structural morphology of the samples was evaluated using scanning electron microscopy (SEM, JEOL, JSM-6300, Tokyo, Japan) and (Seron Technology AIS 2100, South Korea). Transmission electron microscopy (TEM) images were obtained using a Philips-EM-2085 transmission electron microscope with an accelerating voltage of 120.0 kV. The samples were analyzed by X-ray diffraction (XRD) using a Philips X'PERT MPD X-ray diffractometer (XRD). A JASCO FT/IR-680 PLUS spectrometer was applied to record IR spectra using KBr pellets. The BET specific surface areas and BJH pore size distribution of the samples were determined by adsorption–desorption of nitrogen at liquid nitrogen temperature, using Series BEL SORP 18. The compressive strength and compressive modulus of the samples were measured at a crosshead speed of 2 mm/min in an Instron universal testing machine.

2.6. Porosity and water-uptake capacity

The porosity of scaffolds was measured using the liquid displacement method [22]. Hexane was used as the displacement liquid since it was a nonsolvent for silk and could easily permeate through the scaffold without any swelling or shrinkage of the scaffold. Scaffolds were cut into $5 \times 5 \times 1$ mm^3 pieces and placed in a 10 ml cylinder containing a defined volume of hexane (V_1). The volume of hexane and the hexane-impregnated scaffold was recorded as V_2 and acquired after the scaffold was immersed in hexane for 1 h. The volume difference ($V_2 - V_1$) was the volume of the composite scaffold. The volume of the hexane remaining in the cylinder after the removal of the scaffold was recorded as V_3. The quantity ($V_1 - V_3$), volume of hexane within the scaffold, was determined as the void volume of the scaffold. The total volume of the scaffold was $V = (V_2 - V_1) + (V_1 - V_3) = V_2 - V_3$. The porosity of the scaffold (ε) was obtained as follows: $\varepsilon\ (\%) = (V_1 - V_3)/(V_2 - V_3) \times 100$.

Similarly, the scaffolds were immersed into distilled water. The scaffolds were kept in water at ambient temperature for 48 h to ensure water impregnation into the open pores. The water uptake of the porous scaffolds was calculated as Water-uptake $(\%) = (W_w - W_d)/W_d \times 100$, where W_w and W_d represent the wet weight of the scaffold and initial dry weight sponge, respectively. Pore diameters of the scaffolds were obtained by the average pore sizes of 20 pores under the scope of the SEM. The measured porosity percentages, water-uptake capacity and pore sizes of the SF/forsterite composite scaffolds are presented in Table 1. As can be seen, the porosities were decreased with increasing forsterite contents.