Research Article

Initial Study on 3D Porous Silk Fibroin Scaffold: Preparation and Morphology

Yong Zhao,1 R. Z Legeros,2 and Jing Chen1

1The Department of Dental Materials, West China College of Stomatology, Sichuan University, Chengdu 610041, China
2Calcium Phosphate Research Lab, Department of Biomaterials & Biomimetics, New York University College of Dentistry, NY 10010, USA

Address correspondence to Yong Zhao, zhaodt66@163.com

Received 11 November 2010; Accepted 2 December 2010

Abstract Objective. The aim of the present study is to prepare a 3D porous silk fibroin scaffold with a hierachical structure that can meet the demands for bone tissue engineering. Materials and Methods. 3D fibroin scaffold was prepared by the methods of partial dissolution in acid solution and freeze drying fibroin solution. Results. The nets were composed of a mesh of randomly oriented fibers that ranged between 10 µm and 30 µm in diameter. Branchpoints and three dimensional open spaces were distributed throughout the structure with an average pore size of about 177.9 ± 40.0 µm. Conclusion. With the methods of non-woven silk fibroin net preparation and frozen-dried technics, it is possible to prepare a 3D porous silk fibroin scaffold with hierachical fine structure.

Keywords porous scaffold; fibroin; sponge; non-woven net; freeze drying

1 Introduction

Silk fibroin is an important polymer that provides a set of material options for biomaterials because of the impressive biocompatibility, mechanical properties and biodegradability. Silk fibroin has been investigated intensively as biocompatible and mechanically robust biomaterials for bone, cartilage, and ligament tissue engineering.

Porous silk fibroin structures, such as non-woven net, non-woven nanofibers and sponge, have been fabricated by various attempts, such as partial dissolution in acid solution, electrospinning, freeze drying and salt leaching [1,4,5,7]. Fibroin should be investigated more to prepare the structure with a desired shape and a controlled porous architecture for cell growth, tissue regeneration, and vascularization.

The aim of the present study is try to use partial dissolution and freeze drying methods to prepare a 3D porous bioscaffold with a structure consisting of silk fibroin net and silk fibroin sponge.

2 Materials and methods

2.1 Preparation of non-woven silk nets

Raw B. mori silk fibers were boiled for half an hour in a 0.5% Na2CO3, and rinsed thoroughly with water to remove the glue-like sericin proteins surrounding the fibroin filaments and dried in air. Before using in next step, the silk will be dried in an electric drying chamber at 80°C for 6 h. Degummed silk fibers were soaked into test tube containing the solution of 98% formic acid and 0.01 w/v% calcium chloride (material-to-fluid ratio, 1:200) at room temperature. The fiber suspension was shaken for 30 min to achieve homogenous fiber distribution and kept still for 24 h. Finally, the acid solution was evaporated through water bath at 40°C in an aerator, and the resulting non-woven material was repeatedly washed with distilled water to remove any residual salt and vacuum dried [2].

2.2 Preparation of fibroin solution

The degummed silks were completely dissolved after being soaked in a solution of calcium chloride/ethanol/distilled water (1:2:8 mole ratio) at 80°C for 4 h through stirring. The prepared solution was purified by being dialyzed against distilled water for 3 days. The concentration of silk fibroin aqueous solution was calculated by measuring the volume of solution and weighing the remaining solid after drying. The formula for the calculation of the concentration is as follows:

\[ V_2 = V_1 \times C_1 / C_2, \]

where \( V_2 \) is the volume of fibroin solution after evaporation, \( V_1 \) is the volume of fibroin solution before evaporation, \( C_1 \) is the concentration of the fibroin solution before evaporation and \( C_2 \) is the concentration that will be needed in the experiment. At the present experiment, 20 mL of fibroin solution (\( V_1 \)) with a concentration of 2.5 w/v% (\( C_1 \)) was held in a graduated flask. Finally, silk fibroin solution with
various concentrations of 0.75, 1.5, 3, 6, 9 and 12 w/v% were prepared by dilution or evaporation.

2.3 Preparation of 3D fibroin scaffold
The dried non-woven fibroin net was immersed into the fibroin solution with various concentrations under the vacuum of 700 mmHg for 10 min to remove the air from the net. Then the fibroin net was kept soaking in the solution for 24 h before it was taken out of the fibroin solution and transferred into an aluminum vessel. Next, samples were frozen for 6 h at the temperature of $-80^\circ$C and vacuum dried for 48 h in a freeze dryer (LGJ-22, Beijing, China). All samples prepared in different concentrations were tested with SEM (JSM-5900 LV, Tokyo, Japan) in order to find a suitable concentration of fibroin solution to prepare a spongy porous silk fibroin scaffold. To measure the aperture of the porous structure, SEM photographs in bmp file format were tested with image analysis software (SMileView Ver. 2.1). The border of each pore in top layer was measured in the whole picture and the average pore size was calculated.

3 Results and discussion
Usually the concentration of fibroin solution obtained was approximately 2.4–3 w/v% after being purified by dialysis against water for three days. Then it was stirred slowly at 37°C to make it evaporate and concentrate up to a certain concentration according to experiment design. The fibroin solution with the concentration lower than 3 w/v% was light milky white. The liquid would be getting thicker, more white and more viscous along with the increase of concentration. When the concentration increased nearly to 6 w/v%, the solution became too viscous to be suitable for immersing the silk net into the solution. Furthermore, the solution turned into a semisolid gel as a result of protein crosslink when the concentration was up to 9 w/v% [6].

After soaking the net into fibroin solution and being frozen dried, the microstructure of the samples was tested with SEM for each group of different concentration.

Figure 1 shows the gross of silk fibroin net. The cylinder shape of fibroin net was prepared in a test tube. The nets were composed of a mesh of randomly oriented fibers that ranged between 10 µm and 30 µm in diameter. Branch points and three-dimensional open spaces were distributed throughout the structure with an average pore size of about 177.9 $\pm$ 40.0 µm [8]. The individual fibers generally exhibited a smooth surface as revealed in Figure 2A. Three-dimensional scaffolds are required in tissue engineering to support for the formation of tissue-relevant mimics as well as to promote cellular adherence, migration, formation of new extracellular matrix, tissue ingrowth and to foster the transport of nutrients and metabolic wastes [1].

4 Conclusion
With the use of methods to prepare non-woven silk fibroin net and of freeze drying techniques, it is possible to fabricate a 3D porous silk fibroin scaffold with hierarchical fine structure, which may have a potential use as a bioscaffold or other biomaterials.
Figure 2: SEM metallograph of the prepared scaffold by soaking silk net in 3% fibroin solution. A: non-woven silk net. B: 3D structure with non-woven fibroin net and fibroin sponge (×100). C and D: fibroin sponge structure at higher magnification (×300, ×1000).

Figure 3: SEM metallograph of the scaffold samples prepared in fibroin solution with different concentrations. A and B: silk net soaking in 0.75 w/v% fibroin solution. C: silk net soaking in 1.5 w/v% fibroin solution. Original magnification: ×1000.

References