Universidade do Minho

Escola de Engenharia

Semana da Escola de Engenharia
October 24 - 27, 2011

Macro/micro Porous Silk Fibroin scaffolds with Potential for Articular Cartilage and Meniscus Tissue Engineering Applications

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KEYWORDS

Silk Fibroin;Porous Scaffold; Salt-leaching;Articular Cartilage;Meniscus

1. ABSTRACT

This study describes the developmental physicochemical properties of silk fibroin scaffolds derived from high concentration aqueous silk fibroin solutions. The silk fibroin scaffolds were prepared with different initial concentrations (8%, 10%, 12% and 16% (wt%)) and obtained by combining the salt-leaching and freeze-drying methodologies. The results indicated that the antiparallel β-pleated sheet (silk-II) conformation was presented in the silk fibroin scaffolds. All the scaffolds possessed macro/micro porous structure. Homogeneous porosity distribution was achieved in all the groups of samples. The mechanical properties of the scaffolds exhibited concentration dependence. The dry state compressive modulus increased from 0.81±0.29MPa to 15.14±1.70MPa, and the wet state dynamic storage modulus increased around 20-30 folds at each testing frequencies when the silk fibroin concentration increased from 8% to 16%. Based on these results, the scaffolds developed in this study were proposed to be used in meniscus and cartilage tissue engineering scaffolding.

2. MATERIALS AND METHODS

2.1 Scaffold preparation

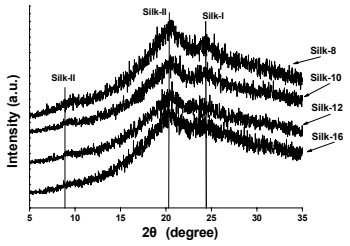
The silk fibroin was firstly dissolved in lithium bromide (9.3 M) solution and then dialysis the solution in distilled water for 2 days. The dialyzed solution was concentrated in poly(ethylene glycol) solution (20 wt%) to get the concentrated silk fibroin solution. The silk fibroin scaffolds were prepared by the addition of sodium chloride particles (500-1000 μm) in silk fibroin solutions of different concentrations (8%, 10%, 12% and 16%, wt%).

2.2 Scaffold Characterization

The scaffolds conformation was confirmed by XRD and FTIR. The morphology was analyzed by SEM. The compressive moduli and dynamic mechanical properties of the scaffolds were screened.

3. RESULTS

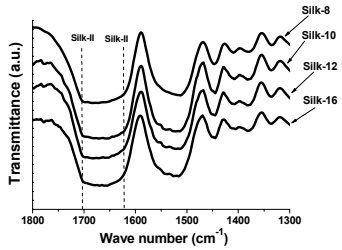
XRD analysis was performed to determine the crystalline structure in the scaffolds (Figure 1). From Figure 1, it is possible to observe that there were no significant differences between the four groups in respect to the peak positions. The peaks at 20.5°-20.8° can be assigned to silk-II based on the previous studies (Kim 2005). All these peaks are broad and of low intensity, which is an indication that the prepared scaffolds possess low crystallinity and uncertain amount of random coil.



Figures 1: XRD Pattern of Silk Fibroin Scaffolds

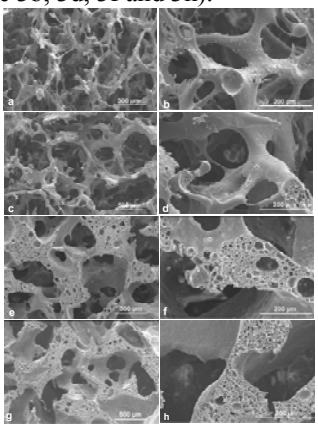
FTIR is also a reliable technique to further confirm the crystal conformation in silk fibroin. Figure 2 shows the FTIR spectra of silk fibroin scaffolds obtained by combining sat-leaching and freeze-drying methodologies. The peaks located at 1701-1704 cm-1, 1622-1627 cm-1 can be attributed to silk-II structure (Kim et al, 2005). The corresponding peak positions of

the main groups are mostly the same for all scaffolds. It should be addressed that the way the FTIR was performed can also affect the final spectral as reported by Demura *et al*. (Demura et al, 1989)



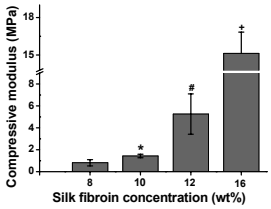
Figures 2: FTIR Spectra of Silk Fibroin Scaffolds

The pores morphology of the prepared silk fibroin scaffolds was investigated under an SEM. From SEM images, mainly two types of pore size were observed among the cross-section of the scaffolds (Figure 3). The morphology of the developed scaffolds varied among the different initial concentrations used. The silk-8 and silk-10 presented branched-like morphology (Figure 3a and 3c), while silk-12 and silk-16 seemed to possess thicker trabecular structures compared to silk-8 and silk-10 based on SEM observation (Figure 3e and 3g). From Figure 3, pores of several hundred micrometers were observed (named L-pore, Figure 3a, 3c, 3e and 3g). There were also pores with size less than 100 μm (named S-pore) distributed inside the trabeculae of the L-pore (Figure 3b, 3d, 3f and 3h).



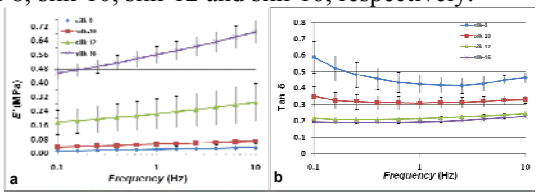
Figures 3: SEM Images of Silk Fibroin Scaffolds

The static compressive modulus of the dried silk fibroin scaffolds increased dramatically as the increase of the silk fibroin concentration. The modulus increased from 0.81±0.29MPa to 15.14±1.70MPa as the silk fibroin concentration increased from 8% up to 16% (Figure. 4).



Figures 4: Compressive Modulus of Silk Fibroin Scaffolds

From DMA data, we can observe that the storage modulus of all the groups increased by the increase of the frequency from 0.1 to 10 Hz, but the increase profiles were different (Figure 5a). The moduli of silk-8 and silk-10 do not increase as distinctly as that for silk-12 and silk-16 scaffolds. During the testing frequency, the moduli were from 12.8±4.2 to 33.7±7.5kPa, 37.6±1.7 to 77.9±4.4kPa, 158.0±16.8 to 264.1±26.8kPa, and 399.2±19.6 to 630.3±49.8kPa for silk-8, silk-10, silk-12 and silk-16, respectively.



Figures 5: DMA Analysis of Silk Fibroin Scaffolds

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