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Facile Preparation of Silk Fibroin Scaffold Via Direct Solvent Exchange*

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Abstract

Although silk fibroin (SF) materials have gained extensive attention in tissue engineering due to their good machinability, biocompatibility, biodegradability, the complex processes, unmatched pore structures, and chemical crosslinker still hinder their mass production and clinic use. In this study, we reported a direct green and high-efficiency process to fabricate 3D silk fibroin scaffold by solvent exchange in water. The pore parameters were easily regulated with NaCl as auxiliary porogen. More importantly, without using any crosslinker or organic chemical-induced crystallin, SF scaffolds is mainly constructed with stable silk II crystalline (constituent with β-sheets), which was confirmed by Flourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). Additionally, the scaffold in our research keeps good tunability on mechanical properties, which has been demonstrated by the results of mechanical testing and provides a feasible way to optimize physical cues for further applications. Thus, the 3D porous silk scaffold with high efficiency and promising structures broaden the potential as a substitute for biomaterials.

Keywords: Silk Fibroin; Formic Acid; Solvent Exchange; Porous Materials

1 Introduction

In tissue engineering, The scaffolds play a significant role to provide three-dimensional templates and synthetic extracellular matrix (ECM) environment for tissue regeneration [1]. In addition to mimic the 3D geometry for tissue engineering, the porous materials should provide a microenvironment for cell attachment, differentiation, and proliferation [2-5]. Therefore, the qualified scaffolds have to meet some attributes, such as chemical composition, physical structure, and biological function.

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Silk fibroin (SF) is a naturally occurring polymer, which has been widely used and investigated for centuries [6]. In particular, SF has been drawing many attention on biomaterial due to its biocompatibility, biodegradability, controllable mechanical properties, and versatility in processing into multiple materials formats [7, 8]. For instance, SF can be easily processed into film, hydrogel, microsphere, and porous material, which has been widely explored as a substitute to repair the defects of native tissues [9].

How to increase surface area and provides a microenvironment for cell attachment and proliferation, as well as nutrient and waste exchange are the main focuses for 3D porous materials [10]. Among so many scaffolds, SF scaffolds have become an ideal substitute for tissue repairing and regeneration. In order to fabricate SF scaffold, various fabrication strategies have been developed, including salt leaching, lyophilization, freeze-thaw treatments, gas foaming, electrospinning, and 3D printing [11-16]. Nevertheless, it is the unmatched characteristics that restrict their application, including the inappropriate microstructures, fast degradation rate, and low mechanical properties. Although 3D printing provides a promising method to fabricate porous materials, the uncontrollable on micrometer level and the unmatched biological ink hinder its applications [17-19]. Additionally, the high cost, organic solvents, and complex process are remaining hinder their widely applied [20, 21]. Thus, it's necessary to develop a new approach for preparing silk porous scaffold.

In this work, we reported a novel and effective method to fabricate 3D silk fibroin porous materials by dissolving silk into formic acid (FA) and salt filtration. As the previous reported [22, 23], silk can be dissolved in FA-Ca^{2+} solution system with simple operation and high dissolution efficiency. Besides, in order to satisfy the purpose that the pore structures can be tuned to match the requirements of applications, we adopt NaCl to adjust the pore morphology. Specifically, the morphology and microstructure of the scaffolds were characterized using scanning electron microscopy (SEM), flourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and the mechanical properties of SF scaffold were measured using the Texture Analyzer. Thus, this work provides a new and facile approach to produce SF scaffold efficiently.

2 Materials and Methods

2.1 Preparation of SF Solution

Regenerated BSF solution was prepared as following the procedure described previously [24]. Briefly, B. mori silk cocoons (Huzhou, Zhejiang, China) were boiled 98 ± 2 °C three times in 0.05wt% Na_2CO_3 for 30 min to remove silk sericin, rinsed thoroughly with deionized water, and then dried at 60 °C oven overnight.

Degummed silk was dissolved in FA-$CaCl_2$ (4.5wt%) solvents, yielding a 18% (w/v) SF-FA-$CaCl_2$ solution[25]. meanwhile, the mixture was diluted with FA to 15%, 12%, 9% (w/v). After being centrifuged at 10 000 rpm for 3 min, the mixtures were sealed and stored at room temperature for further use.

For control groups, the degummed silk was dissolved in 9.3 M LiBr solution, by stirring at 60 °C for 2 h. and fresh silk fibroin solution was obtained after dialysis and filtration, which was stored at 4 °C for further use.