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# Fabrication and Characterization of Spongy Porous Silk Fibroin Materials Crosslinked by Genipin ⋆

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**Abstract**

The porous Silk Fibroin (SF) scaffolds crosslinked by Genipin (GP) were prepared by freeze-drying. The different amount of GP were added in different concentrations of SF solutions. Compared to the uncrosslinked porous SF scaffolds, the GP crosslinked scaffolds were insoluble and showed uniform pores and porosity. The SF scaffolds, with average pore size 39–167 $\mu$m and porosity 49–71%, could be obtained by changing concentrations of fibroin solution, When the concentration of fibroin increased, the pore size diminished. The fourier transform-infrared (FT-IR) results revealed that GP could react with the $-NH_2$ groups on the side chains of SF macromolecules to form inter- and intro-molecular covalent bonds. The FT-IR, X-ray Diffraction (XRD) and Differential Scanning Calorimetry (DSC) results also showed that the crystallinity of SF scaffold crosslinked by GP was increased slightly while the thermal stability was improved. This new fabrication way provided the basis theory for producing scaffolds with reasonable structure.

*Keywords*: Silk Fibroin; Porous Materials; Genipin

# 1 Introduction

In recent years, SF, a naturally occurring polymer that has been used for centuries in the production of textiles and clinical sutures [1], is widely used as a starting material for advanced

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biomedical applications. Regenerated fibroin solution has been fabricated into various material formats, which include films, three-dimensional porous scaffolds, electrospun fibers and microspheres for tissue engineering and controllable drug release applications [2-5]. The impressive cytocompatibility and malleability of SF materials make silk a popular starting material for tissue engineering scaffolds used in skin, bone, blood vessel, ligament, and nerve tissue regeneration [6-8].

Biomaterials required a certain degree of strength and toughness. SF porous materials prepared from SF solution which is obtained after dissolving the natural SF fibers are usually water dissoluble. Therefore it is necessary to adopt crosslinking method to stabilize the SF materials against water before they can be used in tissue engineering. Physical methods include high-temperature processing, methanol/ethanol treatment and ultraviolet radiation [9]. These methods could improve the material's water resistance but may have a negative effect on rigidity and brittleness of the scaffold in the dry state [10, 11]. The common chemical crosslinking agents used in stabilizing SF materials include glutaraldehyde [12, 13], carbodiimide [14, 15], and polyethylene glycol diglycidyl ether (PEGDE) [16]. But these chemical agents may bring different cytotoxicity to the biomaterials, which reduce the biocompatibility and bioactivity of materials. In order to overcome this problem, we here attempted to seek a non-toxic chemical agent to crosslink porous SF materials, intending to decrease water solubility and maintain the biocompatibility.

Genipin, a natural cross-linking agent with lower cytotoxicity, is isolated from the fruits of the plant Gardenia jasminoides Ellis and is obtained from geniposide via enzymatic hydrolysis with $\beta$-glucosidase, which could display desirable characteristics as a naturally occurring crosslinking agent [17]. Genipin has been used to fix biological tissues [18] and to crosslink biomaterials containing amino-group such as collagen, chitosan [19-21] and gelatin [20, 22] with excellent cytocompatibility and tissue compatibility.

In this paper, the GP was employed to prepare SF spongy by freeze drying technique. Also, the performances of the SF scaffolds such as water solubility, morphology and condense structure were analysed by SEM, FT-IR, XRD and DSC. Depending on this study, a new SF based scaffold was fabricated by GP, a naturally green crosslinking agent, which may contribute to the SF based biomaterials construction.

# 2 Materials and Methods

## 2.1 Preparation of Regenerated Silk Fibroin Solution

*Bombyx mori* raw silk fibers were purchased from Zhejiang the Second Silk Co. Ltd. (Huzhou, China). Silk fibroin solution was prepared using a chemical degumming method before dissolution and dialysis. Raw silk fibers were treated three times in 0.05 wt% $Na_2CO_3$ solution at 98 ± 2 °C for 30 min respectively to remove sericin. After being air-dried, the refined silks were dissolved in ternary solvent $CaCl_2$:$CH_3CH_2OH$:$H_2O$ (mole ratio = 1:2:8) at 78 ± 2 °C for 1 h. Then the mixed solution was dialyzed in deionized water for 4 days to get fibroin solution with concentration of about 2.7 wt%. Such solution was again stirred slowly at controlled temperature 37 ± 2 °C to make it evaporate and concentrate. Finally, SF solution with concentration of 4 wt% was prepared.