

# Porous Materials Based on Bombyx Mori Silk Fibroin

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**Abstract:** Bombyx mori silk fibroin (SF) is one of the most important fibers among biomedical porous materials due to its superior machinability, and biocompatibility. It is also chosen for its biodegradability and bioresorbability. It is a protein-based fiber. In this paper, we have reviewed the key features of SF. Moreover we have focused on the morphous, technical processing and biocompatibility of SF porous materials. We have also dealt with its application in research. Finally, a perspective on potential applications and problems of SF porous materials were provided.

**Keywords:** Bombyx mori silk fibroin, porous materials, processing, biomaterial, application, biocompatibility, biodegradation.

## 1. Introduction

Porous three-dimensional materials and the network structure materials are composed of interconnected or closed pores. They possess some excellent characteristics, such as favourable mechanical properties and optic-electrical properties, good permselectivity, selective adsorption and chemical activity. Porous three-dimensional biomaterials provide a microenvironment for attachment, increase surface area, support a large cell mass, form an extracellular matrix and play an important role in manipulating cell functions in regenerative medicine [1]. Moreover, tissue engineering is an interdisciplinary and multidisciplinary field that aims at the development of biological substitutes that restore, maintain, or improve tissue function [2].

Bombyx mori silk is a naturally occurring polymer that has been used in textile production and as clinical suture for centuries [3]. Silk in its natural form is composed of a filament core protein, SF, and a glue-like coating consisting of a family of sericin protein. SF consists of heavy (H) and light (L) chain polypeptides of ~390 kDa and ~26 kDa, respectively, linked by a disulfide bond at the C-terminus of the two subunits, and associates with the H-L complex primarily by hydrophobic interactions [4]. The hydrophobic blocks tend to form  $\beta$ -sheets or crystals through hydrogen bonding and hydrophobic interactions, forming the basis for the tensile strength of SF [5]. These ordered hydrophobic blocks combine with the less ordered hydrophilic blocks to give rise to the elasticity and toughness of SF [6]. SF also exhibits diverse structures, mechanical properties and biocompatibility. Based on these features, interest has

arisen in the use of Bombyx mori SFs as starting materials for biomaterials and scaffolds for tissue engineering [7]. Recently, there have been many reports about SF porous materials which have been widely investigated in controlled drug delivery system, anticoagulant blood materials, biosensors, artificial ligaments, artificial tendon and artificial skin, etc [8]. In this paper, we will focus on SF based porous materials derived from Bombyx mori silkworms. Several preparation methods, different morphous of SF porous materials and its applications are reviewed as follows.

## 2. Silk fibroin processing

### 2.1 Solution of silk fibroin

Because SF is coated by sericin, degumming is very important. High-purity SF fiber can be obtained easily from degummed silk [9]. SF can be dissolved in neutral solutions of salts such as LiSCN, LiBr, and  $\text{CaCl}_2$  [10,11]. In the processing of SF porous biomaterials, preparation of SF-based scaffolds with high porosity and interconnected homogeneous pores has become one of the major challenges. Several methods including salt leaching, freeze-drying, gas forming and freeze-drying/foaming have been developed to fabricate porous fibroin scaffolds [12].

### 2.2 Non-woven silk fibroin mats

Non-woven SF nets/mats/membranes can be prepared using SF with diameters in the range of several to tens of micrometers in their native or partially dissolved forms [13]. A process for producing non-woven SF

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nets/mats/membranes comprises the following steps [14]: firstly, degumming and removal of the sericin; secondly, by a homogenization and drying step (Figure 1) that yields 3D, nonwoven nets/mats/membranes. Non-woven mats can also be obtained by electrospinning SF fibers with different diameters [15]. Electrospinning uses electrical forces to produce polymer nanofibers with diameters around fifty nanometers and arbitrary length. Electrospinning occurs when electrical force at the surface of a polymer solution or melt overcome surface tension and viscoelastic forces and create an electrically charged jet. When the jet dries or solidifies, an electrically charged fiber remains, which can be directed or accelerated by electrical force and then collected in sheets or other useful shapes [16]. The two methods as mentioned above are the predominant ways to obtain SF-based porous mats.

## 2.3 Silk fibroin hydrogels

Hydrogels are three-dimensional polymer networks which are physically durable for swelling in aqueous solution but do not dissolve in these solution [17]. Hydrogels are formed from regenerated SF solution by a sol-gel transition in the presence of acid, ions, or other additives [18]. During the gelation process, SF experiences a structural transition from random coil to  $\beta$ -sheet due to enhanced hydrophobic interactions and hydrogen bond formation [19, 20]. The processes for producing regenerated SF hydrogels are as follows: a) silk is obtained from silk cocoons; b) the sericin layer covering the silk fibers is removed; c) the disulfide bonds are broken in order to obtain aqueous SF solutions; d) the silk aqueous solutions are concentrated; e) some acid, ions, or other additives are added; f) after further processing, such as freeze-drying, microporous SF sponges are formed from hydrogels. Recently, many applications suggest the potential of porous hydrogels for cell culture and in regenerative medicine [21]. Those will be reviewed in later sections.

## 2.4 Silk fibroin porous sponges

Porous sponges are important tissue engineering materials. SF porous sponges can be obtained using porogens, gas forming, and freeze-drying, freeze-drying/foaming, electrospun fibers [22]. Solvent-based sponges were prepared using salt (e.g., sodium chloride) or sugar as porogen. Porogens such as NaCl were added into SF aqueous solutions in disk-shaped containers, and then the containers were covered and left at room temperature for 24 hours, and then leaching the salt in water at room temperature for 24 hours and drying it [1]. Freeze-drying-based sponges were prepared using crosslinking agent, freezing and drying [9]. The freeze-drying/foaming method was a composite processing method to prepare 3-D SF scaffolds. Unlike the freeze-drying method, the ice/silk composites were firstly placed in the atmosphere at 20°C for different times to make them partly thaw and then lyophilized leaving a porous material [12]. Electrospinning has also been one of the important methods to obtain SF porous scaffolds. There are many ways to prepare the 3-D SF scaffolds. Those mentioned above does not cover all the ways, but rather the predominantly used methods to produce the material.

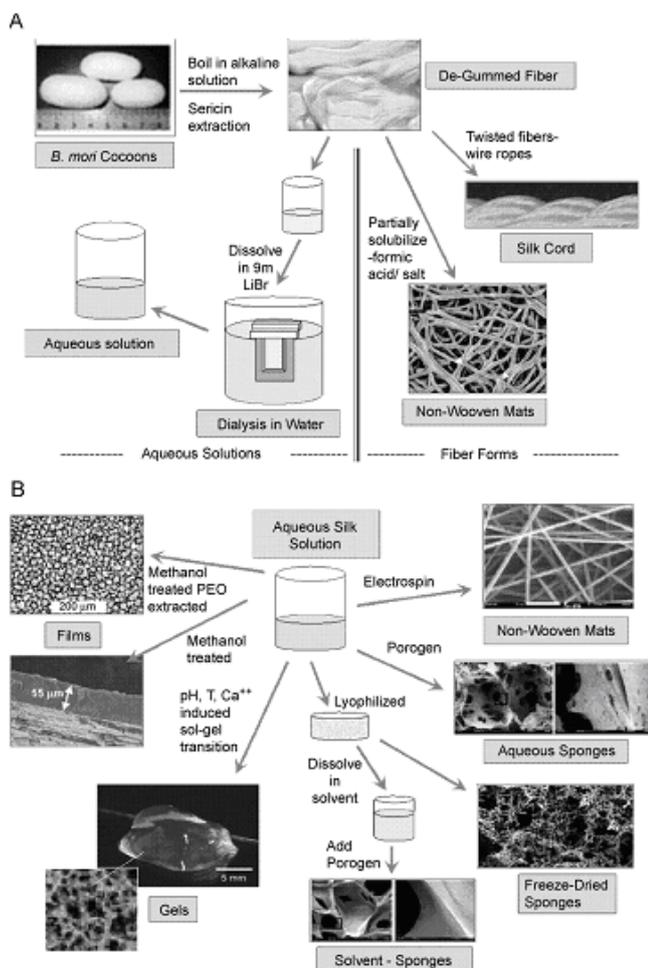


Figure 1. (A) Silk fibroin is purified from sericins via boiling in an alkaline solution. (B) Processing of silk morphologies from aqueous silk fibroin solution [17].

### 3. Biocompatibility and degradation of silk fibroin porous materials

As a biomaterial, the “heterogeneity” or immunogenicity of a SF porous biomaterial is a crucial limitation for its clinical applications. Once a heterologous antigen enters the body, B cells [23], macrophages, dendritic cells [24] and mast cells [25] from our immune system are activated and produce antibodies and various cytokines targeting antigen epitopes on the biomaterials to attack and get rid of the “foreigners” by humoral and cellular immune responses. The biocompatibility of SF porous materials is an important first consideration. Several primary cells and cell lines have been successfully grown on different SF porous materials to demonstrate a range of biological outcomes. SF porous materials are biocompatible when studied *in vitro* and *in vivo* [26, 27]. SF nonwoven nets may be excellent candidates for clinical applications since they both enjoy a long-lasting biocompatibility, inducing a quite mild foreign body response, but no fibrosis, and efficiently guide reticular connective tissue engineering. In recent years, some researchers have studied the immunogenicity of SF biomaterials. The residual sericin plays a crucial role affecting the biocompatibility of SF porous materials. Small fibroin particles and soluble sericin protein extracted from native silk fibers did not induce significant macrophage activation [7]. While sericin did not activate macrophages by itself, it demonstrated a synergistic effect with bacterial lipopolysaccharide. The *in vitro* inflammatory response of degummed SF compared with polystyrene and poly (2-hydroxyethyl methacrylate) shows less adhesion of immunocompetent cells [28]. SF films [29] implanted *in vivo* and SF non-woven mats implanted subcutaneously in rats induced a lower inflammatory response. Surface modification is also an important method to improve the performance of SF porous materials. For example, surface modification with the integrin recognition sequence (Arg-Gly-Asp) RGDs can increase cell attachment [30]. Glucose-oxidase was immobilized on SF films for use as a glucose sensor [31], and so on.

Biodegradation behaviours of SF porous materials play an important role in regenerative biomedicine. Many *in vitro* and *in vivo* studies have shown that the degradability of SF porous biomaterials was related to the mode of processing and the corresponding content of  $\beta$ -sheet crystalline form [17]. A useful scaffold for tissue engineering materials should be biocompatible, as well as biodegradable [32]. Li *et al.* [33]

investigated the degradation behavior of porous SF sheets by *in vitro* enzymatic experiments with  $\alpha$ -chymotrypsin, collagenase IA, and protease XIV. Rebecca *et al.* [34] researched SF yarns' degradation. Results support that silk is a mechanically robust biomaterial with predictable long-term degradation characteristics by many detection methods. Wang *et al.* [35] implanted 3-D SF porous scaffolds into two kinds of rats and observed the morphology of the materials at different times. Gu *et al.* [36] have investigated the degradation behaviors of SF-nerve guidance conduits (SF-NGCs) *versus* SF fibers. The results collectively indicated that SF-NGCs were able to degrade at a significantly increasing rate to meet the requirements of peripheral nerve regeneration. These results demonstrate that the *in vivo* behavior of the three-dimensional SF scaffolds is related to the morphological and structural features that resulted from different scaffold preparation processes.

In summary, the *in vitro* biocompatibility and degradability of SF porous material has been proved. An important characteristic of SF is an increasing instability and solubility over time *in vitro* and *in vivo*, due to enzymolysis. Long-term stability and mechanical integrity are essential for cells that require sufficient time and stiffness to produce their tissue-specific matrix. Therefore, it is necessary to adjust degradation rate of the SF scaffolds material in order to match with the tissue regeneration.

### 4. Application of silk fibroin based porous materials

Recently, SF porous material has been investigated in biomedical materials fields including bone and cartilage, skin tissue, vascular grafts, nerve repairing, ligaments and tendons. In addition to all the mentioned above, SF porous material was also studied in repairing of cornea, wound dressings, drug release, sensors and so on. All of these aspects exhibit the great prospects of SF porous materials in biomedical applications.

#### 4.1 Bone and cartilage

Bone tissue is a specialized form of connective tissue, which is composed of calcified extracellular matrix and bone cells including osteoprogenitor, osteoblasts, osteocytes and osteoclasts. The bone matrix consists of both an organic and inorganic matrix [37]. The biodegradability, distinguishing mechanical properties, and low inflammatory response of SF [38] ensure its role as one of the promising porous materials for

osteogenic applications. Recently, SF porous material has been the primary biomaterial observed as bone and cartilage materials.

In vivo implantation of electrospun Bombyx mori SF fibers in calvarial defects in mice facilitated the complete healing of the defect with new bone within 12 weeks [39]. Similarly, Bombyx mori SF hydrogels have been used as scaffolds for bone tissue growth both *in vitro* and *in vivo* in rabbits without inflammatory effects [40]. Aside from biologically generated bone as above, options to control hydroxyapatite mineralization on silk biomaterial matrices have also been reported [41]. The results suggest increased osteoconductive outcomes with an increase in initial content of apatite and BMP-2 in the SF porous scaffolds. The premineralization of these highly porous SF protein scaffolds provided enhanced outcomes for the bone tissue engineering [42]. Calcium-phosphate (Ca-P) coatings have been shown to reduce the fibrous encapsulation layer and enhance direct bone contact and stimulate differentiation of bone marrow stromal cells along the osteogenic lineage [43]. Survival rate of seeded cells is very critical for bone tissue engineering, but the seeded cells inside the scaffold may not survive sufficiently to repair a large critical sized defect. These cells like recruiting host cells to participate in new bone formation and defect repair [44-47]. Moreover, highly porous scaffolds which performed the role of a temporary matrix for anchorage dependent cells are an important factor in the success of tissue engineering. Vascularization which is also critical for osteogenesis, seeded cells, as well as resident host cells is essential to repair critical sized defects successfully.

## 4.2 Skin tissue

Skin tissue is the biggest organ in human's body. It plays a crucial role in protecting human body from the environment, dehydration, and infectious agents. Several studies show that SF porous material can accelerate wound healing, improve adhesion and spreading of normal human keratinocytes and fibroblasts, upgrade the growth and development of skin tissue. Proteins are among the most successful materials applied as skin grafts [48]. Fibrin is a kind of good skin substitute [49].

Some people carried out a series of studies to investigate the potential of SF matrices and SF blend matrices for improving the skin repairing [50-53], and the outcomes demonstrated the strong potential of SF or SF blend matrices as skin regeneration substitutes. In our studies, SF porous materials were prepared by

freeze-drying. The L929 cells can attach and proliferate well in the SF porous materials, and it was similar to that in collagen materials. No signs of cellular lysis, intracellular granulation or cell morphological changes were observed. In conclusion, SF porous materials have great potential in skin tissue repairing. But reduction of the scar and regeneration of coil gland are still some problems that need to be resolved in skin repairing.

## 4.3 Vascular grafts

SF porous matrices have potential applications as vascular graft matrices due to their ability to support the attachment, proliferation and differentiation of vascular cells and resist shear stress and pressure from simulated blood flow.

Many attempts have been made to develop small-diameter blood vessels due to increasing demands for vessel transplants, but all these approaches have almost failed. Greatly reduced graft patency was observed when cell-free synthetic prostheses were utilized for small diameter arteries, such as coronary and infragenicular vessels [54]. Bondar *et al.* [55] have investigated endothelial cell (EC) responses to nano- and micro-scale silk fibers in terms of cell morphology, proliferation, formation of intercellular contact, and expression of adhesion molecules. Outcomes revealed no significant differences between micro- and nanofibrous scaffolds. Also, interactions between ECs and SF matrices were investigated through the expression of specific transmembrane receptor molecules. The results of real-time PCR revealed significant up-regulation of integrin- $\beta_1$  in ECs grown on nanofibrous compared to microfibrillar scaffolds. Soffer *et al.* [56] have developed SF into porous tubular structures. The average burst strength of the tubular scaffolds was greater than those prepared with collagen [57]. However, further development is needed to reach the gold standard of the saphenous vein whose burst strength is very large [58]. Following Soffer's work, the evaluation of the biological potential of these electrospun SF porous matrices for vascular grafts was determined [59]. The proliferation, metabolic viability, morphology and phenotype of human aortic endothelial cells (HAECs) and human coronary artery smooth muscle cells (HCASMCs) on 2-D electrospun SF porous matrices were examined. The results show that electrospun SF porous matrices have good biocompatibility. Thus, the need for further research into both the biological and mechanical properties was demonstrated. Future work with SF small-diameter vascular grafts will need to focus on co-cultures of

endothelial and smooth muscle cells in a tubular, perfusion environment to more closely mimic the *in vivo* environment.

#### 4.4 Nerve grafts

Peripheral nerve repair represents a common clinical challenge, and the current gold standard for treating large nerve defects involves the implantation of nerve auto-grafts that is limited by graft availability, secondary deformities, and potential differences in tissue structure and size [60]. It has been previously reported on good *in vitro* biocompatibility of SF fibers with peripheral nerve tissues and cells [61]. Several authors have reported that nerve conduits (NC) releasing neurotrophic factors can enhance nerve regeneration across long nerve gaps [62]. The SF graft was used for bridge implantation across a 10-mm long sciatic nerve defect in rats, and the outcome shows that SF grafts could promote peripheral nerve regeneration with effects approaching those elicited by nerve autografts which are generally considered as the gold standard for treating large peripheral nerve defects [63]. However, involving the implantation of nerve auto-grafts, research gold standard for treating large nerve defects is still endeavored. For example, the methods of preparation of nerve material, some new starting material obtained for nerve material, the interaction between material and organism and so on.

#### 4.5 Drug delivery

Drug delivery technologies were used in modifying drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance [64]. However, many medications such as peptides and proteins, vaccines, and gene based drugs, in general, may not be delivered using conventional routes. Bombyx mori SF materials for drug delivery may improve the drug delivery situation. And SF is a protein soluble in water, and, when processed into scaffolds, resulting in a biomaterial with excellent mechanical properties, slow bio-degradation and well established biocompatibility. So, SF has been suggested as a platform for drug delivery either in the form of films [65] or as genetically engineered silk-elastine hydrogels [66] and other SF or SF blended materials [67-70].

Because SF protein might be susceptible to enzymatic degradation or cannot be absorbed into the systemic circulation efficiently due to its molecular

size and charge issues. For this reason, we still have many problems in further research. Current efforts in the area of drug delivery include the development of targeted delivery in which the drug is only active in the target area of the body (for example, in cancerous tissues) and sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation. Types of sustained release formulations include liposomes, drug loaded biodegradable microspheres and drug polymer conjugates.

#### 4.6 Other application studies

Besides all those mentioned above, SF porous materials were also studied in some other fields. For example, glucose oxidase was immobilized onto the blended membrane of poly (vinyl alcohol) (PVA) and regenerated into silk fibroin. The morphology and application to glucose biosensor were investigated [71]. Moreover, SF porous materials were regarded as cornea material [72], tracheal scaffolds [73], ligaments and tendons [74] and so on. In summary, application research of SF porous material demonstrated that it has great potential to be used in biomaterial field.

### 5. Further prospects

With the development of SF porous materials, more and more novel SF porous materials will be fabricated. However, there are still some problems which should be solved:

- (1) SF can be processed into diverse morphologies to meet the different needs. Novel methods should be originated to create a wide variety of exciting SF porous materials.
- (2) Biodegradation rate should be controlled and made to match with the growth of an organism.
- (3) Surface modification also should be used to improve biocompatibility of SF porous materials. To develop SF porous biomaterials for clinical usage, the materials with lower immunogenicity or without the immunogenicity should be taken into consideration.
- (4) To ensure that more and more new and promising SF porous materials could be applied in clinical medicine.

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