Preparation of Silk Fibroin/Cellulose Blend Films

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Abstract. Regenerated silk fibroin (SF) has excellent biocompatibility and degradability, but its mechanical properties need to be improved. As the most widely distributed and most abundant polysaccharide in nature, the advantage of cellulose material is its good strength and modulus. In this study, SF was dissolved in formic acid and calcium chloride solution. Then glycerol and ultrasonic microcrystalline cellulose were added, and the SF/cellulose blend film was prepared by the delayed flow method. The properties of the blend films were characterized by a series of tests such as electron microscope, FTIR, tensile strength, and so on. The results showed that when the ratio of cellulose to SF reached 30 to 70 and the addition of glycerol was 30% of the total solute, the properties of SF/cellulose blend films were the best. The addition of micro-nano-cellulose fiber improved the mechanical properties of the blend film, and the addition of glycerol greatly improved the flexibility of the blend film, which expanded the application of SF/cellulose blend films in the field of biomaterials.

1. Introduction
Silk is a natural fiber secreted by the silk glands of the silkworm, which is composed of two proteins, silk fibroin and sericin wrapped in silk fibroin [1]. SF, as the main component of silk, plays a decisive role in its mechanical properties. SF has good biocompatibility and degradability, so many kinds of cells can survive, add value, or adhere to SF materials [2,3]. SF, as a scaffold for tissue engineering, can be slowly absorbed by the body and eventually metabolized into peptides and amino acids. These properties determine that it has a very good application prospect in the biomedical field, such as surgical suture, wound dressing, tissue engineering scaffold, and so on [4-6]. Also, when silk is made into nanofibers by modern science and technology, the excellent properties of silk fibers will be brought into full play in the biomedical field. To sum up, SF is a kind of biomedical material with great potential, which provides more possibilities for biomaterials.

As a very important natural renewable material, cellulose is the most abundant and widely distributed polysaccharide in nature [7]. Cellulose has a wide range of sources, degradability, renewability, low cost, and excellent mechanical properties. Compared with other biomaterials, cellulose nanofibrils (CNFs) has the characteristics of biodegradability, low pathological toxicity, supporting cell adhesion, and proliferation [8,9], so CNFs is more suitable to be used in the biomedical field than other biomaterials. Various studies have shown that CNFs can promote the production of globulin in human cells, so it can be used in the treatment of wound healing and reducing inflammation in vivo [10-13].

As far as the internal structure of silk is concerned, its comprehensive mechanical properties are much higher than those of other polymer materials [3,16]. However, after degumming, dissolution, and
re-molding, the internal orientation of silk will be seriously reduced and its molecular weight will be lost, which will greatly reduce its mechanical properties and affect all its properties. Therefore, it is difficult for regenerated SF to be widely used in the biomedical field. Previous studies have shown that the mechanical properties of regenerated SF scaffolds are significantly enhanced after mixed reinforcement of short fibers. Besides, chemical modification is often used to enhance the mechanical properties of SF materials. But its efficiency is low, so we usually use the former to enhance the mechanical properties of SF films. That is, it is mixed with some natural polymers with good mechanical properties, such as polyvinyl alcohol (PVA), cellulose, chitosan, and so on [2,14].

Therefore, it is assumed that the regenerated SF solution can be blended with cellulose fiber to enhance the mechanical properties of SF films. Combined with its biodegradability, it can be used in many biomedical fields. The blend films have excellent flexibility and fatigue resistance and can be used as biomaterials for heart valves or medical dressings.

2. Materials and methods

2.1. Preparation of SF solution and cellulose

* Bombyx mori * raw silks were degummed by the established procedure described previously. Briefly, Bombyx mori raw silk fibers (Huzhou, China) were boiled three times in 0.05wt % Na₂CO₃ for 30 min to remove sericin, rinsed thoroughly with deionized water, and then dried in an oven at 60°C for 24 hours. The degummed silk was dissolved in the mixed solution of formic acid (Shanghai, China) and CaCl₂ (Shanghai, China) to obtain a regenerated silk fibroin solution. The CNFs (Shanghai, China) was placed in the ultrasonic cell grinder with a power of 500W for 10 minutes to obtain the cellulose after ultrasonic treatment.

2.2. Preparation of SF/Cellulose blend films

The preparation process of the SF/cellulose blend membrane is shown in Figure 1. Glycerol (Shanghai, China) and microcrystalline cellulose after ultrasonic treatment were added to the regenerated SF solution. The evenly mixed emulsion was quickly spread out in a disposable petri dish and air-dried in a fume cupboard for three days. After drying, the SF/cellulose blend film was obtained. Among them, the content of glycerol is 30% of the total solute mass. According to the mass ratio of cellulose (CEL) to silk fibroin (SF), the samples were labeled as SF/CEL=100/0, SF/CEL=80/20, SF/CEL=70/30, and SF/CEL=60/40.

![Figure 1](image)

Figure 1 Schematic representation of a strategy for SF/cellulose blend films.

2.3. Characterization of SF/Cellulose blend films
The morphological of the SF/Cellulose blend films were observed by SEM (Hitachi, S4800, Japan). Their structure analysis was characterized by the FTIR (Bruker, Vertex70, Germany). Mechanical properties were performed by INSTRON 5967, electronic universal material testing machine, with chuck sensor force 1KN, tensile speed 10mm/min, clamping distance of 30mm, spline width10 mm, and sample dimensions 10mm × 50mm. The thickness of the film was measured 5 times with a spiral micrometer to get the average value, and each sample was tested at least 3 times to get the average value.

3. Result and discussion

3.1. Morphology of SF/Cellulose blend films

![SEM results of SF/Cellulose blend films surface and cross-section. (A1) Pure SF films surface, (A2) Pure SF films cross-section, (B1) SF/Cellulose blend films surface, and (B2) SF/Cellulose blend films cross-section](image)

It can be seen from the figure that the surface (Fig. 2 A1) of the pure SF film is smooth, the cross-section (Fig. 2 A2) is flat, and the SF membrane is relatively complete as a whole because the addition of glycerol improves the flexibility of the SF film to a great extent so that the SF film will not dry and crack because it is too brittle in the process of air drying. The surface (Fig. 2 B1) of SF/cellulose blend film is rough, and many protrusions can be seen, which is due to the addition of cellulose fibers. It can be seen from the cross-section (Fig. 2 B2) that there is no obvious phase separation in the blend membrane, indicating that the cellulose fiber can be uniformly distributed in the SF membrane.

3.2. Structure analysis of SF/Cellulose blend films
As shown in Figure 3, the four groups of SF/CEL=100/0, SF/CEL=80/20, SF/CEL=70/30, SF/CEL=60/40 blend films all have an absorption peak near 1635 and 1523 cm\(^{-1}\), corresponding to amide I and amide II in turn. Amide I is mainly C=O stretching vibration, C-N stretching vibration and N-H bending vibration, while amide II is mainly N-H bending vibration and C-N stretching vibration [15]. So, their secondary structures are all β-folds. This shows that compared with the pure silk fibroin membrane, the composite material of silk fibroin and cellulose does not form a new chemical bond, so there is a physical blending between silk fibroin and cellulose.

### 3.3. Mechanical Properties of SF/Cellulose Blend Films

![Mechanical Properties of SF/Cellulose Blend Films](image)

**Figure 4** Mechanical Properties of SF/Cellulose blend films. (I) Stretching in dry state, (A) SF/CEL = 100/0, (B) SF/CEL = 80/20, (C) SF/CEL = 70/30, (D) SF/CEL = 60/40; (II) Stretching in wet state, (a) SF/CEL = 100/0, (b) SF/CEL = 80/20, (c) SF/CEL = 70/30, (d) SF/CEL = 60/40.

Tensile tests were carried out to evaluate the mechanical properties of SF/cellulose blend films. In the dry state (Fig. 4I), the tensile strain of pure silk fibroin film (curve A) is larger, up to 80%, and the elastic modulus is higher. But its initial modulus is low, that is, the tensile strength is low. When a certain
amount of cellulose fiber was added (curve B), the initial modulus and tensile strength of the film increased significantly. Although the tensile strain has been reduced to a certain extent, about 60%, it is still in a relatively superior position. With the increase of the content of cellulose fiber (curve C), the initial modulus of the film increased continuously, the tensile strength reached 5Mpa, and the tensile strain changed little. At this time, the strength and elasticity of the film reached a peak. As the cellulose content continued to increase (curve D), the elastic modulus and initial modulus of the membrane began to decrease, and the strength and elasticity of the membrane were not as good as those of pure silk fibroin membrane. The reason for this phenomenon is that the mechanical properties of SF/cellulose blend films are mainly determined by silk fibroin, the content of silk fibroin accounts for most of the blend films, and the addition of a small amount of cellulose improves the mechanical properties of the blend membranes, played a role in strengthening. However, when the content of cellulose is too high, the content of silk fibroin which provides mechanical properties is too low, even if the cellulose fiber plays a role of reinforcement, it cannot make up for the impact of too little silk fibroin on the blend film, so the mechanical properties of the blend film will decrease.

In the control group without (curve C'), the tensile strain of the blend film was only 20%, while that of the blend film with glycerol was 60%. The comparison between the two groups showed that the addition of glycerol greatly improved the elasticity and flexibility of the blend film.

In the wet state (Fig. 4II), the elastic modulus of the blend film is generally higher, and the strength of the blend film decreases. When the ratio of silk fibroin to cellulose is 70/30, the mechanical properties of the blend films are the best, which is consistent with the tensile test in the dry state. The blend membrane with excellent mechanical properties can be used for the preparation of tissue engineering scaffolds such as heart valves and vascular stents.

4. Conclusions
In this study, SF/cellulose blend membranes were prepared successfully. The electron microscope pictures show that the cellulose fibers can be uniformly distributed in the SF membrane. FTIR results show that SF and cellulose are only physical blends, and the addition of cellulose does not change the secondary structure of SF. The tensile test showed that the mechanical properties of SF films were enhanced by the addition of cellulose, and the flexibility of the blend films was improved by the addition of glycerol. When SF/cellulose = 70/30, the mechanical properties of the blend films reached the best. This study provides the possibility for the further application of regenerated SF in the field of tissue engineering.

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