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The Flexible Silk Fibroin Films Modified by Genipin and Glycerol

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The silk fibroin (SF) films, modified by genipin (GP) and glycerol (Gl), with favourable mechanical properties, were obtained by a casting/solvent evaporation method. Simultaneously, the chemical, mechanical and structure properties of the films were examined and analyzed. Compared to uncrosslinked SF films, fibroin solubility of the modified SF (MSF) and Gl/MSF films in the warm water decreased dramatically from 46 % to 15 %, which exhibited good stability under physiological environment. The best valuable modified films with tensile strength at 18.0 MPa, breaking elongation at 171.1 % and Young's modulus at 463.1 MPa were obtained when the GP and Gl content were 20 wt. % and 20 wt. % relative to the amount of fibroin. The deformability of the MSF films augmented significantly with increasing of Gl concentration. Fourier transform-infrared (FT-IR) results revealed GP could react with SF macromolecules to form inter- and intra-molecular conjugated covalent bonds. Moreover, the FT-IR, X-ray diffraction (XRD) studies illustrated the GP induced conformational transition from random coil to β-sheet of SF chains, yielding MSF and Gl/MSF films with enhanced stable thermalbility. The cytocompatibility of the MSF films were evaluated through MTT assay using L929 fibroblast. Compared to the SF films treated with 75 % ethanol, the MSF films exhibited significant cytocompatibility, which was demonstrated by cell adhesion, proliferation and cell morphology. The intrinsic properties and biological results suggested that the MSF films may be potential a candidate material for wound dressing applications or tissue engineering strategies.

Introduction

The soluble silk fibroin solution can be obtained by treatment with reagent such as $CaCl_2$-EtOH-H_2O, $Ca(NO_3)_2$-MeOH-H_2O or aqueous LiBr [1] and purified by dialysis against deionized water. SF exhibits its advantages in excellent biocompatibility, thermal stability, oxygen permeability and low inflammatory response. Previous reports illustrated that the SF materials had been utilized for wound protection, drug delivery system, peripheral nerve regeneration materials, blood vessel engineering, bone tissue engineering and organic electronics. [2-7] The SF molecules show the characteristics of random coil conformation in aqueous solution, with an average hydrodynamic radius of 139 nm. Besides, its molecular weight of heavy chain and light chain are ~300 kDa and ~26 kDa respectively. [8] Normally, the SF films can be prepared by casting the SF solution onto different substrates, such as polytetrafluoroethylene, polyethylene, polystyrene, or glass. These films can be dissolved easily in water or solvents without additional treatment. Generally, stable SF films can be obtained by means of physical methods, including high-temperature processing, methanol/ethanol treatment and ultraviolet radiation. [9] Though these methods are useful in stabilizing the SF film against water, the β-sheet-rich films are generally rigid and brittle in the dry state, causing difficulty in practical applications. In order to improve both stability and mechanical properties of the SF films, chemical reagents can also be adopted by crosslinking or blending. Experiment process normally implemented by the participation of bi-functional reagents, such as glutaraldehyde (GTA), [10] carbodiimides [11] and polyethylene glycol diglycidyl ether (PEGDE). [12] Nevertheless, it is noticeable that chemical reagents would be probably toxic if they were released into host as due to biodegradation. Accordingly, it is necessary to meet the increasing demand for a crosslink agent to form stable and biocompatible crosslinked products without added cytotoxicity problems. GP is a naturally crosslinking reagent with lower cytotoxicity, which can be isolated from the fruits of the plant Gardenia jasminoides Ellis and is obtained from geniposide via enzymatic hydrolysis with β-glucosidase. [13] A series of studies indicated GP had been adopted as a reagent for repairing biological tissues [14] and crosslinking biomaterials containing amino groups such as collagen, chitosan and gelatine, [15-20] These products possessed higher cytocompatibility and stability. On the basis of former studies, Gl, a kind of small molecule polyalcohol, can be used as plasticizer to improve properties of films with non-toxic to human body. The

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films blended with Gl performed modified mechanical properties, especially the elongation at break. [21] The Gl can reduce phase separation between silk and PVA in the blend. [22] These earlier studies substantiated that blending Gl directly was beneficial for improving the properties of fibroin films.

Various silk-based materials such as film, sheet, and scaffold have been prepared to satisfy tissue engineering applications through different ways. Unfortunately, these silk materials produced from regenerated fibroin solution lose the flexibility in the dry state, which generated more practical difficulties in some applications. In this paper, we utilized GP and Gl to prepare novel flexible, stable and less cytotoxicity SF films. Those films characterized mechanical properties of tensile strength, elongation at break, and Young's modulus by mechanical test, solubility in aqueous media by measuring fibroin solubility, conformational structures by FT-IR and XRD, thermal properties by Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), and biocompatibility by MTT assay.

Experimental

Materials

Bombyx mori raw silk fibers were purchased from Zhejiang the Second Silk Co. Ltd. (China). GP were purchased from Wako Pure Chemical Industries, Ltd. (Japan), methyl thiazolyl tetrazolium (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Company (USA). L929 cells, Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS) and penicillin/streptomycin were purchased from Shanghai Pu-fei Bio-Technology Co. Ltd, (China). All other chemicals used in this study were purchased from Shanghai Sinopharm Chemical Reagent Co. Ltd. (China) without purification.

Preparation of regenerated silk fibroin solution

Silk fibroin solution was prepared using a chemical degumming method before dissolution and dialysis. Raw silk fibers were treated three times in 0.205 wt % Na_2CO_3 solutions 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 3 wt %.

Preparation of silk fibroin films

The modified SF solution was prepared by adding the GP directly to the 3 wt % SF solution in various weight ratios of 0, 5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, and 30 % w/w GP/SF. The Gl groups were also prepared by mixing the 20 % w/w GP/SF solution and Gl in various weight ratios of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 % w/w Gl/SF. Then 40 mL mixed solutions were gently shaked in the thermostat at 37 °C for 12 h, poured separately into polystyrene plates of 10 cm × 20 cm and dried at 60 °C for 2 h. All the films were removed from the mold after keeping them at room temperature for 12 h. SF films without GP and Gl used as control materials and were prepared according to the process described above.

Mechanical tests

Stress-strain curves of strip shaped (120 mm × 5 mm, thickness around 50μm) films equilibrated at constant relative humidity of 65 % for 24 h were performed on an INSTRON Testing Machine 3365 at standard conditions (20 °C and 65 % relative humidity), and a crosshead speed was 20 mm/min. The thickness of the samples was determined using a Mitutoya thickness equipment ID-C112BS. The Young's modulus E, was performed with a Y391 Yarn Elasticity Tester. Elongation value was obtained after a drawing load of 0.735 N was exerted on the samples for 5 secs. The experiment was performed under the following conditions: pretension of 0.2 N and gauge length of 50 mm. The initial tensile modulus was calculated according to the Eq. (1)

$$E = \frac{P \times L_0}{S \times \Delta L} \quad (1)$$

Where E is initial tensile modulus (MPa), P is drawing load (N), L_0 is specimen gripping length at pretension (mm), S is specimen cross-section area (mm^2) and ΔL is elongation value in 5 s (mm). The measurement was repeated three or more times and averaged.

Degree of crosslinking

The degree of crosslinking of the MSF films was determined by ninhydrin assay. [23] The films were weighted (50mg, n =3 per group) and heated with ninhydrin solution (2 wt. %) at 100 °C for 20 min. The number of free amino groups in the sample was proportional to the absorbance of the solution. The absorbance of the resulting solution was recorded at a wavelength of 570 nm using a Bio-Tek Synergy HT microplate reader. Glycine solutions of various known concentrations were used as standards, and SF films without GP were used as control materials. Then we can calculate the degree of crosslinking (D, %) by following Eq. (2):

$$D = \frac{(NH_2)_{UC} - (NH_2)_C}{(NH_2)_{UC}} \times 100\% \quad (2)$$

Where $(NH_2)_{UC}$ and $(NH_2)_C$ represent the mole fraction of free NH_2 remaining in uncrosslinked and crosslinked samples, respectively.

Stability of fibroin films

Stability of fibroin in the modified films was assessed through measuring the weight loss of the films in the warm water. Take 100 mg of films baking in 105 °C to constant weight measure, and calculate the moisture content w (%). Then under the same condition measure the materials weight m_1 (mg), deionized water was injected into tapered bottle with 1:100 g ml^{-1} bath ratio, take each material into the water, after shaking the materials at 37 °C for 24 h, filtered, dry remains of the films at 105 °C, and measured the weigh m_2 (mg). Fibroin solubility (S, %) in the water was determined by Eq. (3):

$$S = \frac{(m_1 - m_1 \times w) - m_2}{m_1 - m_1 \times w} \times 100\% \quad (3)$$

Characterization

FT-IR spectroscopy

The SF films were cut into micro-particles with radius less than 40 μm, and then the samples were prepared in KBr pellets. For each measurement, each spectrum was obtained by a Nicolet Avatar-IR360 with the wave number ranging from 400 to 4000 cm^{-1}.

XRD

XRD was performed by a Rigaku D/Max-3C diffractometer with Cu-Kα radiation (λ = 0.15418 nm). The X-ray source was operated at 40 kV and 40 mA. Diffraction intensity was measured in reflection mode at a scanning rate of 2 °/min for 2θ = 5~40 °.

TGA and DSC

TGA and DSC measurements were performed with Perkin-Elmer DSC-7. The temperature was lowered to room temperature and increased to 500 °C at a heating rate of 10 °C min^{-1}. Sample weight was 3-4 mg. The open aluminum cell was swept with N_2 during the analysis.

Cell culture and MTT assay

L929 cells were maintained in complete DMEM medium with 10 % FBS and 1 % penicillin/streptomycin as described earlier. [24] All the samples were cast on 24-well tissue culture plate wells and irradiated with an exposure does of 20 kGy by Co60 irradiator followed by extensive washing with sterile PBS (pH 7.4) prior to cell seeding. Each sample was made in triplicate. Cells were trypsinized, counted, and plated at a density of 5×10^4 cells/well into films, and grown for 1, 3, 5, 7 and 9 days. The plates were transferred in a 37 °C and 5 % CO_2 incubator in 95 % humidity and the medium was changed every second day. The 24-well plate without materials and the uncrosslinked fibroin films treated with 75 % ethanol were used as blank and positive controls respectively. On specified days, cell viability was evaluated using MTT assay. In brief, 100 μl of MTT (5mg/mL) diluted 1:10 times in PBS was added to each well followed by incubation for 4 h at 37 °C. After incubation, 1 mL of dimethyl sulfoxide was added to dissolve the blue formazan product formed and the absorbance (OD values) was measured at 595 nm using a Bio-Tek Synergy HT microplate reader.

Results and discussion

Mechanical properties

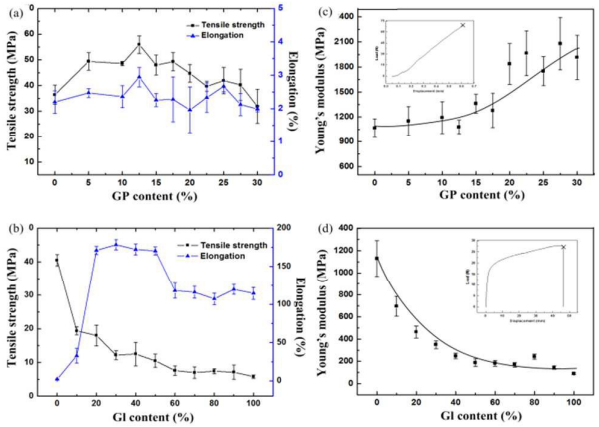

Figure 1. Effect of GP and Gl content on mechanical properties of MSF films (a) tensile strength and elongation with different GP content, (b) tensile strength and elongation with Gl content, (c) Young's modulus with different GP content, and (d) Young's modulus with different Gl content.

Tensile testing of the films provides a pattern on the strength and elasticity of the films given by the parameters like tensile strength, elongation at break and Young's modulus. Mechanical values properties provide the information about the inherent character of the films. A value of moderate tensile strength, low elongation at break, high Young's modulus suggests a polymeric film is hard and brittle; a value of moderate tensile strength, high elongation at break, low Young's modulus suggests a polymeric film is a soft and tough, and a hard and tough polymeric film is characterized by high elongation at break, Young's modulus and tensile strength. [25] A strong and elastic film in nature could expand the range of application in biomaterials.

To elucidate the effect of concentration of GP and Gl on the physical properties of SF films, tensile measurements were carried out. Figure 1 showed the tensile strength, elongation and Young's modulus at the break of MSF films with different GP content and Gl/MSF films with different Gl content in the dry state respectively. The typical stress-strain curves of the MSF films with 20 % GP and the Gl/MSF films with 20 % Gl were embedded in the Fig. 1 (c) and (d). The tensile strength of the uncrosslinked SF films was weaker than the MSF ones. The changes of mechanical tensile strength among different GP percent concentrations indicated that mechanical strength increased first and then decreased a little by increasing GP content in films. Conversely, the adding of GP enhanced Young's modulus gradually (Figure 1 (c)). The Young's modulus and tensile strength of the MSF films crosslinked by 12.5 wt. % GP were 1078.5 ± 83.3 and 55.9 ± 3.4 MPa respectively, which were tougher than the other films, but the films still exhibited brittle in the dry state. Figure 1 (b) and (d) showed increasing Gl levels resulted in a sharp decrease in tensile strength, a rapid increase in the elongation at break and a decline in Young's modulus of the Gl/MSF films. At about 20 wt. % Gl, the Young's modulus, tensile strength and elongation at break of Gl/MSF films were 463.1 ± 54.4, 18.0 ± 3.1 MPa and 171.1±5.3 % respectively, which became more soft and kept its tensile

strength at the same time. Those results showed the small amount of Gl made MSF film turned to flexile from brittle. A suitable amount of GP added to the SF solution leaded to chemical reaction between SF molecular chains.[23] While the covalent bond with cyclic structure connected fibroin chains in the system improved the regularity in the films, which result in increase of the tensile strength, but can't change the brittle property in the films. However, excess rigid crosslinking sites made films fragile and prone to cracks and flaws. As a result, introduction of Gl could overcome the disadvantage of brittle materials. Many studies indicated that the characteristic property of other polysaccharide or protein films were improved by plasticized with different hydrophilic compounds.[26, 27] Since addition of Gl, the hydroxyl groups of which interact with SF molecular chains, replacing water molecular in silk fibroin chain hydration and leading to larger gaps among SF molecules.[21] So the softness and elasticity of the films increased due to molecular chains movement became easy. What's more, the increased Gl content enhanced the hydrophilic properties in the films, which could form more bound water rather than free water from environment to augment plasticity in nature. However, the elongation of the Gl/MSF films decreased a little at more than 60 % Gl, may be ascribed to reduction of random coil structure in the films followed by decrease the area of hydration between Gl and SF. The results above indicated that adding GP around 15 wt. % and Gl at 20 wt. % was effective in improving the mechanical properties. This MSF films with tough charateristic are found to be suitable for biomaterials application.

Stability of fibroin films

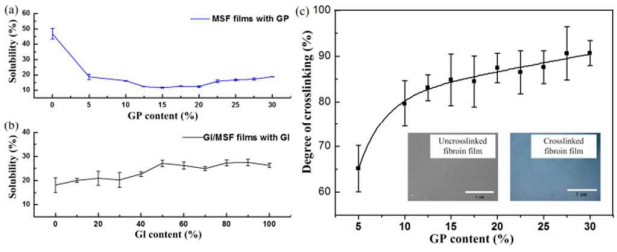

Figure 2. Fibroin solubility of (a) MSF films with GP, (b) Gl/MSF films with Gl, (c) degree of crosslinking.

The fibroin solubility of the MSF films and Gl/MSF films (with varying levels of GP and different Gl content) in the warm water is shown in Figure 2 (a) and (b). Without GP, very high solubility of the uncrosslinked silk fibroin was observed (46 %), with only 5% GP (MSF films), the solubility dropped dramatically to 19 %, and the mass loss stabilized at 15 % GP and above. The amount of fibroin solubility was found to be dependent on the GP content in the films. These results suggested that the GP greatly stabilized the films against aqueous solvent. Compared to the uncrosslinked SF films, fibroin solubility of the Gl/MSF films also dropped dramatically with addition of Gl. Additionally, the addition of Gl didn't have obvious influence on those films. But slight increase of solubility was observed in Figure 2 (b) when Gl content exceeded 20 wt. %. The trend of increase may be ascribed to the loss weight of superfluous Gl which can't attach the SF molecular through hydrogen bond. In order to test the formation of covalent bonds in fibroin films, the degree of crosslinking under different conditions were evaluated using ninhydrin assay (Figure 2 (c)). After 3 h of reaction a color change in the solutions was observed from light yellow to light blue, indicating the reaction between SF chains and GP. With the extension of reaction time, the fibroin solution eventually turned to be dark blue and the crosslinked films with maximum crosslinking degree was obtained as shown in the Figure 2. Many studies described that GP reacts with amino acids or proteins to form dark blue pigments associated with the oxygen-radical polymerization of GP.[17] The higher crosslinking degree related with stable films obtained when the GP content reached 12.5 wt. %. Crosslinking reaction markedly reduced the fibroin solubility degree of the SF films. The MSF films with crosslinking degree of above 85.08 ± 2.85 % exhibited stability under the simulated physiological environment.

Conformational structures analysis

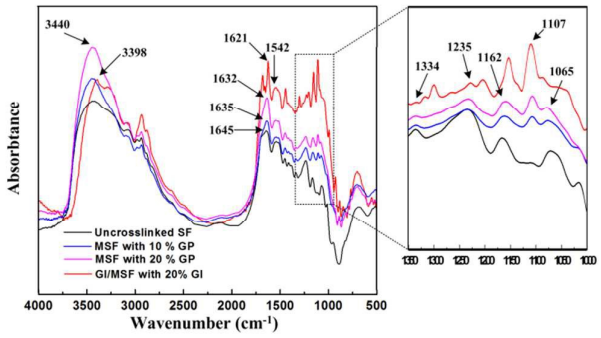

Figure 3. FT-IR spectra of uncrosslinked SF and MSF films

FT-IR spectroscopy could characterize the secondary structure of proteins by examining the absorptions in the range 4000-500 cm-1. FT-IR of uncrosslinked SF and MSF films modified by GP and Gl in the range of 4000-500 cm^{-1} and partial enlarged drawing were represented on Figure 3. SF protein exists in three conformations namely random coil, Silk I (α-helical) and Silk II (β-sheet conformation). The strong absorptions at the 1645 cm^{-1} for amide I (C=O stretching), 1542 cm^{-1} for amide II (N-H deformation), 1235 cm^{-1} for amide III (C–N stretching, C=O bending vibration)) were observed from the uncrosslinked SF film group (black line), which indicated it contained random coil and α-helical conformations.[28, 29] The spectra of the MSF films (10 wt. % and 20 wt. % GP) showed shifts in the absorptions band of amide I from 1645 cm^{-1} to the lower wave number 1635 cm^{-1} depicted the silk the β-sheet structure in the MSF films after crosslinked by GP.[29] The amide I and amide II bands shifted to 1623 cm^{-1} and 1230 cm^{-1} respectively in the red line, intensities of these shifted peaks increased proportionally, illustrating augment of the β-sheet structure. Meanwhile, there appeared new absorption bands at 1107 cm^{-1} (-CHO) among all the MSF fims, which further verified new covalent bonds appeared among silk fibroin molecules. 23 Moreover, adsorption band appeared at 1235 cm^{-1} (amide III) represented a mixed vibration of CO-N and N-H. Owing to decrease of the $-NH_2$ on the lysine after reaction, this

adsorption band and characteristic adsorption bands at 1334 cm^{-1}, 1162 cm^{-1}, and 1065 cm^{-1} decreased after the addition of GP. The SF and MSF films showed a peak at 3400 cm^{-1}, upon blending with GI, the band of the films at 3400 cm^{-1} shifted to a lower wave number. Displacement of the peak can be associated with an interaction between fibroin and GI. Moreover, the intensity at 3400 cm^{-1} decreased with addition of GI, which can be explained that there had been an increase in the specified intermolecular hydrogen bonds between SF chains.

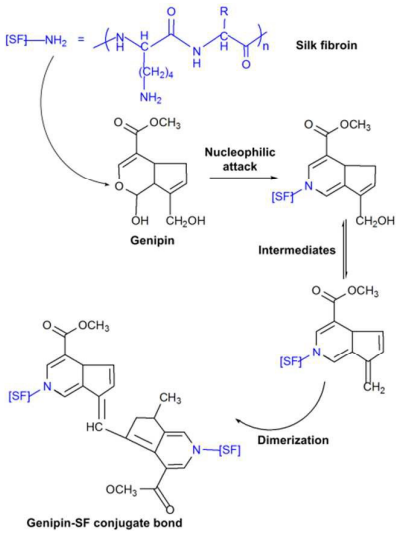

Figure 4. Predicted mechanism schematic of the reaction

The GP crosslinking of MSF films might induce conformational changes due to the structural rearrangement of chains to form covalent bonds. Some studies confirmed that GP crosslinking of SF is followed by protein conformational changes. [23] The exact mechanism behind the chemical action between SF and GP hasn't been fully described. But it is similar to that observed for amino-group containing compounds, [17] where amino groups of SF interact with the ester groups of GP, leading to the formation of secondary amide linkages. The predicted reaction mechanisms of SF crosslinked by genipin may be similar to the previous reports [17] and illustrated in Figure 4. At first, the amino groups in the SF initiate nucleophilic attacks at C-3 of six-membered ring, resulting in the opening of the GP dihydropyran ring followed by formation of a nitrogen-iridoid (intermediates). And then GP-SF intermediates self-polymerization occurs by radical reaction of two amino-attached rings, which created highly conjugated heterocyclic genipin derivatives. SF proteins contain amino acids lysine and arginine, which could react with GP. Although the fraction of these amino acids is very low, increasing amount of GP ensures the action take place. The FT-IR results confirm the crosslinking reaction. The basic sequence of the crystalline block is of hydrophobic group $–(Ala–Gly)_n–$ that adopts a β-sheet structure and resulted in higher hydrophobicity. [30] The introduction of the GI enhanced hydrophobic interactions in silk fibroin molecules, further leading structure transformation from random coil to β-sheet. It may be concluded that crosslinking reaction taken place between SF molecular and GP induce conformation changes in the MSF films. Increasing of covalent bond and β-sheet structure gave the MSF films attractive mechanical properties and stability.

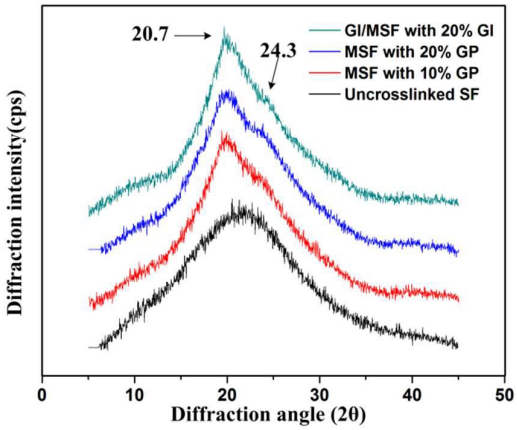

Figure 5. XRD diffraction curves of uncrosslinked SF and MSF films

The conformation transformation of MSF films was further investigated using X-ray diffraction curves of the different SF films samples (Figure 5). The uncrosslinked SF film showed arc-shaped scattering at around 20° and weak diffraction at around 12.2°, corresponding to the random coil and α-helical structure. There appeared to be a obvious characteristic peak at 20.7° in the MSF films with GP content equal 10 wt. %, assigned to β-sheet structure. [31] With the increasing of GP content, the red and blue curves showed strong and sharp diffraction at around 20.7° and weak diffraction at around 24.3°, illustrating that the content of β-sheet conformation increased. GI/SF films showed a broad peak between 9.1°~12.2°, which was attributed to the superposition of the two kinds of crystallization peak. Furthermore, compared to uncrosslinked SF films, the peak at 20.7° showed much stronger intensities, this result investigated that crosslinking reaction could induce the formation of β-sheet conformation from random coil and/or α-helical structure, which was consistent with the results of FT-IR spectra.

Thermal analysis

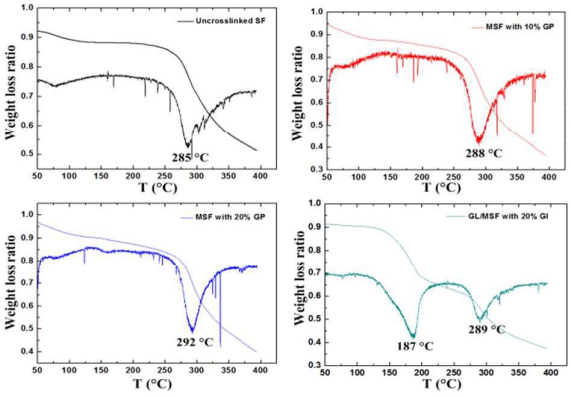

Figure 6. TG and DTG curves of uncrosslinked SF and MSF films

Thermal analysis could reflect the interaction among SF, GP, and Gl. Thermal stability of different kinds of films was investigated using TG weight loss and DSC curves. The TG weight loss curves and differential coefficient curves were shown in Figure 6. The major weight loss temperature (T_m) started at 285 °C for the uncrosslinked SF films, and at 288, 292, 289 °C for the 10 wt. % GP crosslinked SF films, 20 wt. % GP crosslinked SF films and 20 wt. % Gl modified SF films with 20 wt. % GP respectively. These results showed that the MSF films exhibited higher thermal stability than uncrosslinked SF films because the crosslinking reaction and increase of β-sheet. The weight loss within 150-200 °C may originate from the loss of bound water molecules due to the addition of Gl.

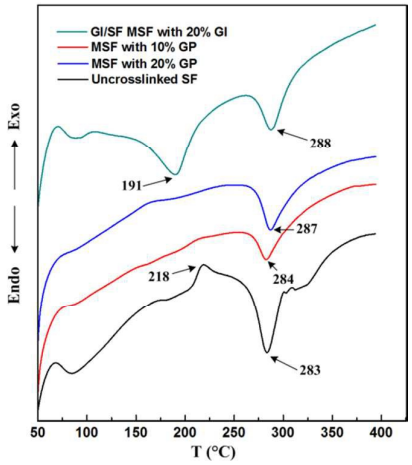

Figure 7. DSC curves of uncrosslinked SF and MSF films

Figure 7 showed the DSC of the different SF films samples. The DSC curves for the uncrosslinked SF films showed one large endothermic peak at around 283 °C (T_d), attributed to thermal degradation of fibroin. [32] The uncrosslinked SF films (black line) was quite different from the other samples in the presence of an exotherm transition at 218 °C, which was ascribed to the strong molecular motion within transformation of random coil to the β-sheet structure during the heating process. [32] The thermal behavior of uncrosslinked SF films was typical of an amorphous SF with random coil conformation as previously shown by FT-IR results. The MSF films crosslinked by GP showed significant reduction in intensity of exotherm peak, which indicated the changes in the films from random coil to β-sheet after GP crosslinking. The increase in the thermal stability, given by the increase in Td, was related to the increase in the extent of covalent crosslink. This fact is another confirmation of the crosslinking reaction between GP and SF. A new endothermic peak at 191 °C appeared in the Gl/MSF films (green line), which maybe caused by degradation of hydrogen bond water molecules, which was consistent with TG resultes. The thermal analysis results certificated increase of structure regularity and thermal ability in the MSF films.

Biocompatibility of SF films

The cell viability and proliferation on films in different days are indicative of the cellular compatibility and appropriateness for tissue engineering applications. [33]

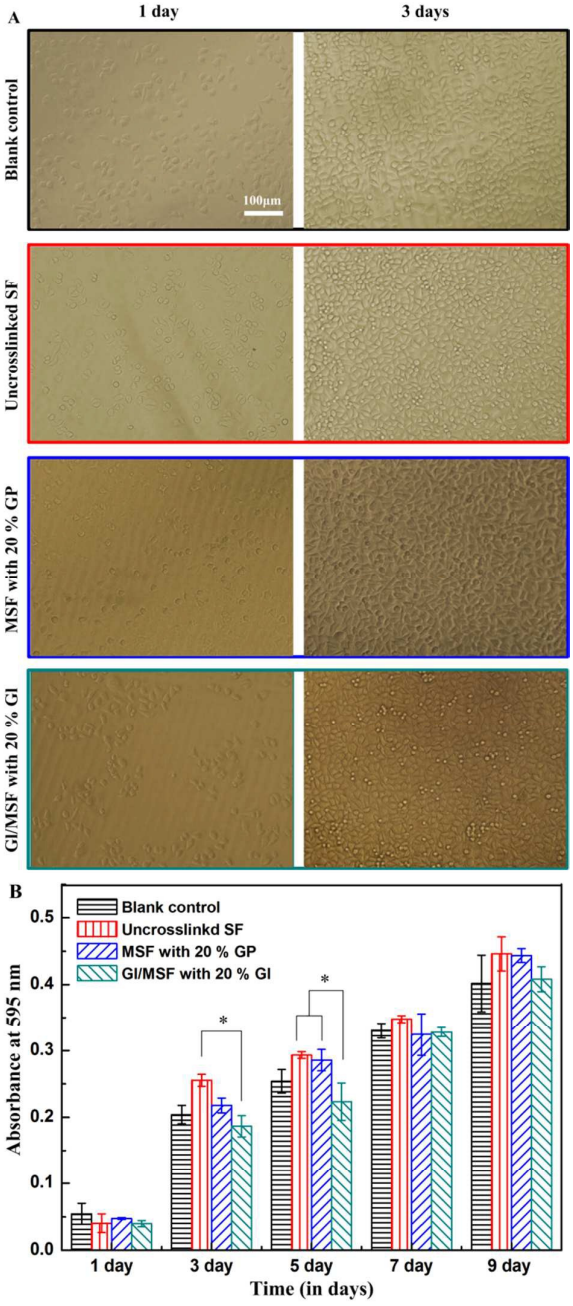

Figure 8. (A) Micrographs of L929 cells on blank plate, SF films, MSF films and Gl/MSF films for 1 and 3 days . (scale bar, 100 μm). (B) Cell viability of L929 cells cultured on blank plate, SF films, MSF films and Gl/MSF films 1, 3, 5, 7, and 9 days. Each point represents the mean ± SD (n=3). * means significant difference between two groups at $p < 0.05$.

In this study, MTT assay was carried out as a preliminary approach to assess the biocompatibility of SF films. Morphology of L929 cells on the films and MTT results of days 1, 3, 5, 7 and 9 in different films were shown in Fig. 8. Compared to the blank control, both uncrosslinked and modified SF films can support L929 cells attachment and

proliferation, because fibroin polymers has been extensively considered as non inflammatory and highly biocompatibility for various cell types. [34] The morphology of L929 cells attached to the films displayed obvious differences at 3 days, which exhibited scalene triangle shape as same as blank control. MTT results showed the number of cells on all the films increased as a function of culture time, more importantly, the cellular viability of cells in MSF films with 20 wt. % GP didn't show a significant decline compared to uncrosslinked SF films and blank control. The OD values of the uncrosslinked SF films and Gl/MSF films was significant difference, the Gl/MSF group had less cells than the pure one after culture on day 3 and 5, however, this significant difference disappeared after 7 days. The observed tiny difference in OD values was probably due to release of surplus Gl in the films. The result suggested that the modified films exhibited good cytocompatibility and supported growth of L929 cells as comparable to uncrosslinked SF films, thus providing good substrate for cell culture.

Conclusions

The objective of this work was to prepare SF films with flexibility, stability and biocompatibility by modified with GP and Gl, and the effect of different amount of GP and Gl on mechanical properties, secondary structure, thermal ability and Biocompatibility was determined. The following conclusions can be drawn from the present investigations.

(1) Excellent mechanical properties of SF films can be obtained through tuning amount of GP and Gl. When the fragment of GP and Gl were 20 wt. % and 20 wt. %, a flexible silk film was achieved with the the Young's modulus at 463.1 ± 54.4 MPa, tensile strength at 18.0 ± 3.1 MPa and 171.1±5.3 % respectively.

(2) As a result of new covalent bonds formed and augment of β-sheet structure in the MSF and Gl/MSF films after GP crosslinking, all the MSF films exhibited an obvious decrease of fibroin solubility in simulated physiological environment and a slight increase of thermal stability. These MSF films also facilitate the attachment and proliferation of L929 cells.

Considering expanding applications of fibroin based biomaterials, the present study provides an effective way to fabricate the flexible, stable and less cytotoxicity MSF films in vitro, which would further propel the applications of various tissue engineering applications.

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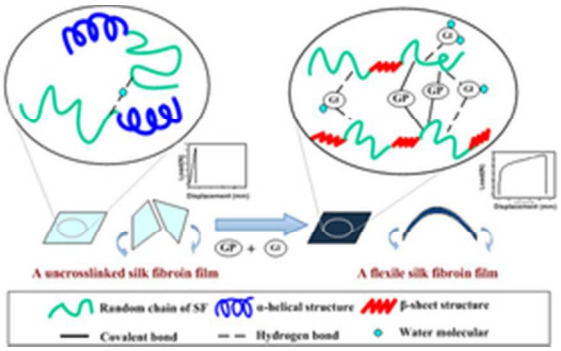

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