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Silk fabric protection obtained via chemical conjugation of transglutaminase and silk fibroin reinforcement

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Abstract

Historic silk fabric is an important part of Chinese precious cultural heritage and its protection has always been a major challenge. This paper proposes a bio-safety method by the chemical conjugation of transglutaminase (TGase or TG) and sodium caseinate (SC), which produced a macromolecular polymer between protein molecules and enhanced silk fabrics. The changes of the mechanical properties of the reinforced silk fabric after washing by 10 cycles were not obvious, indicating good washing durability. After TGase and SC reinforcement, the silk fibroin (SF) solution was sprayed on the surface of silk fabric to improve the mechanical properties, where the secondary structure were formed by the self-assembly of SF to improve the mechanical properties. Therefore, the breaking stress attained the maximum value when the SF solution concentration was 1.0%. Meanwhile, the breaking stress increased by about 20.89% compared with untreated silk fabric. When the artificially alkali aged silk fabric is reinforced, the breaking stress and strain of the reinforced sample increased by 37.77% relative to the alkali aged fabric. The surface morphology and secondary structure transformation of the samples were also analyzed by scanning electron microscopy and Fourier transform infrared spectroscopy, respectively. The results indicated that a significant SF layer was introduced on the surface of the silk fabric and the β-sheet structure increased due to the synergetic role of the macromolecular polymer and SF. Moreover, it is concluded that an increase in temperature and humidity will result in a decrease in the preservation index, which caused the degradation of silk fabric and proved that the preservation time of the reinforced silk fabric in the same environment was longer than that of the unreinforced sample. The biological enzyme chemical conjugation with silk fabric and physical combination of the pure SF solution is expected to be applied to the protection and enhancement of silk cultural relics.

Keywords

silk fabric, sodium casein, transglutaminase, silk fibroin, reinforcement

Exquisite and luxurious silk is a treasure in the long expanse of Chinese history. It is a witness to human civilization and a symbol of ancient culture in China. Raw silk, a protein fiber, is composed of silk fibroin (SF; about 75%) and sericin (about 25%) and contains 18 kinds of amino acids.[1–3] However, silk fabrics are highly susceptible to damage by heat, light, water, and microorganisms[1,4–11] due to their protein properties and special secondary structure.[12,13] Furthermore, the preservation and restoration of genuine silk fabrics excavated from tombs has become a formidable challenge, and most of their structures have been carbonized and degraded due to the influence of different geographical environments.[6,14–17]

In order to determine the appropriate treatment for the storage and display of ancient textiles, various methods have been developed to protect and reinforce the ancient silk fabrics, including adhesive,[18] film,[19,20] raw material supply,[5,21–24] and enzymatic[25–30] and

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hydrophobic coating.[31,32] The selection of cultural relic protection materials should not affect the cultural relics themselves, and many materials have been applied to the protection of silk fabrics. Ahmed and Ziddan[33] designed a new metallic frame to fix textile artifacts, which is useful for the display of museum exhibits, but does not protect against harmful substances. Hansen[18] used parylene-C for the reinforcement of fragile, porous, or fibrous artwork, which increases the breaking strength of the fabric, but the coating hardens under ultraviolet (UV) irradiation. Meanwhile, the coating is a high molecular polymer, and the reversible protection of the silk product is still a challenge. In recent years, researchers have favored the use of biological methods to reinforce historical silk. SF has excellent mechanical properties[34] and is used in many fields, such as biological tissues[35,36] and fluorescence detection.[37] Huang et al.,[21] Wu et al.,[23] and Yang et al.[24] proposed a new consolidation system using SF and ethylene glycol diglycidyl ether (EGDE) for the reinforcement of fragile silk fabrics. This method has been shown to improve the mechanical properties of artificially aged silk fabrics, but the crosslinking agent needs to be considered for its degradation products. Wu et al.[19] prepared a bacterial cellulose membrane to enhance the physical properties of the treated silk fabrics. This bioprotective method is harmless to the fabric, but it takes a long time to cultivate strains. Many researchers[25–28] have adopted enzymatic reactions to reinforce historical textiles, all of which have achieved remarkable results. The transglutaminase (TGase) enzyme,[38–40] which is mostly used in food protein modification, can catalyze the intramolecular and intermolecular crosslinking of protein polypeptides, thereby improving the structure and function of proteins. In addition, more studies[41–48] have shown that TGase can promote a stable biopolymer by covalent crosslinking with sodium caseinate (SC), which improves the mechanical properties of the SC membrane. Zhu et al.[29,30] explored the condition of TGase-mediated thermal aging silk reinforcement, and the results showed that the recovery effect was significant. In addition, from the practical point of view, the fabric is also prone to being affected by UV irradiation.[49] In particular, because the reinforced silk fabric will finally find its utility in common daily life, the topics of biocompatibility and nontoxicity should be considered. Therefore, a simple, harmless, and widely feasible method for silk fabric surface fabrication is desired for facile control of surface reinforcement.

Hierarchical nanoporous structure and crosslinking, which have multi-scale roughness, have been considered as an alternative solution to the abovementioned problems. In this work, we present a bioenzymatic technology using the chemical conjugation of liquid TGase and SC to enhance the mechanical properties of the silk fabric. Thereafter, the SF solution is sprayed on the surface of the fabric treated with TGase and SC to further reinforce the silk fabric. In order to verify the protective effect of the method, the silk fabric is aged by an alkali solution. The nature of the reinforced silk fabric is investigated by the tensile techniques, chromatic aberration, scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR), in order to characterize the surface and inner structure. In addition, the preservation index (PI) is evaluated for the life of the enhanced silk fabric. This safe and harmless bioprotection method is believed to provide a reference for the reinforcement of historical silk fabrics, and the double protection of SC crosslinking inside and SF crosslinking outside the silk fabric has a potential application in the protection of historical silk.

Experimental details

Materials

White silk fabric (130 g/m^2) with five three-flying satin patterns was purchased from Hangzhou Mengjin Silk Co., Ltd (Hangzhou, China) and the yarns per 10 cm in the weft and warp directions were 1220 and 460, respectively. The silkworm cocoon was obtained from a Sericulture farmhouse. TGase (120 units/mL) was kindly supplied by Shanghai Qingrui Food Technology Co., Ltd (Shanghai, China). SC was purchased from Luancheng County Farming Trading Co., Ltd (Shangqiu, China). Phosphate-buffered saline (PBS) was provided by Rui Chu Biological Technology Co., Ltd (Shanghai, China).

Extraction of pure silk fibroin solution

The silkworm cocoon was dissolved in 9.3 M LiBr solution at 60°C with a bath ratio of 1: 6 for 4 hours. After being dissolved completely, the solution was poured into a dialysis bag (molecular weight 3500) and the dialysis bag was immersed in deionized water (replaced every 4 hours) for 3 days. The extracted SF solution was obtained.

TGase and SC treated silk fabric

Five various concentrations of bath temperature (20°C, 30°C, 40°C, 50°C, and 60°C), reaction time (0.5, 1, 1.5, 2, and 2.5 hours), TGase (0.5, 1, 1.5, 2, and 2.5 mL), and SC (0.5%, 1%, 1.5%, 2%, and 2.5%) were used in the experiment to obtain the optimum effect on the breaking stress and strain of silk fabric. In our study, raw silk fabrics were washed with ethanol and acetone firstly, then immersed in 10 mM PBS buffer solution (pH = 7) containing TGase and SC with a bath ratio of 1: 25 in a water bath. The fabric was then placed in deionized water (1:25) for 10 minutes to remove the residual liquid.

Silk fibroin sprayed onto silk fabric

The TGase and SC treated silk fabrics was evenly sprayed with the SF solution at 0.5%, 1.0%, 1.5%, and 2.0% on the fabric surface at a distance of 20 cm from the silk, respectively, which were recorded as 0.5%, 1.0%, 1.5%, and 2.0%, respectively. Samples treated with TGase and SC were not sprayed with SF solution and were noted as 0.0%. All specimens were conditioned at 20°C and 65% relative humidity (RH) for 2 days.

Alkaline and thermal aging of the fabrics

The silk fabrics were immersed in a 5% NaOH solution at 35°C for 9 hours to get artificial aged fabrics. The reinforced silk fabrics were exposed to 125°C, 135°C, 160°C, and 180°C heating for 24 hours in an oven.

Measurement of the fabrics

The mechanical properties of the silk fabrics were measured by an electronic fabric strength tester (HD026N+, Nantong Hongda Experimental Instrument Co., Ltd, China) with 50 mm gauge length and 100 mm/min stretching speed at 20°C and 65% RH. The standard of the test refers to GB/T 3923.1-2013, and the actual size is (100 mm × 30 mm) due to experimental conditions. The warp direction of the fabric was selected to measure the tensile strength.

The fabrics were washed with a wash fastness tester (SW-8 A, Nantong Hongda Experimental Instrument Co., Ltd, China). The standard of the test refers to AATCC 61-2006, and the actual size is (100 mm × 30 mm).

Color differences caused by reinforcing material were characterized using a computer color measurement spectrophotometer (Datacolor 650, Datacolor, USA). The average value was measured four times with an accuracy of less than 0.1, which was a parameter under control of DCI software. Accuracy was related to uncertainty, which needed to be expanded by the coverage factor and uncertainty types A, B, and C. We assumed that the color difference was evenly distributed and calculated the uncertainty according to the following equations

$$u_A = \sqrt{\frac{\sum_{i=1}^{n} (\overline{x_i} - x)^2}{n(n-1)}} \tag{1}$$

Table 1. Detailed test data of the silk used in formulas (1)–(4)

x_1	x_2	x_3	x_4	A	k
92.80	92.82	92.88	93.07	0.10	1.70

$$u_B = \frac{A}{\sqrt{3}} \tag{2}$$

$$u_C = \sqrt{u_A^2 + u_B^2} \tag{3}$$

$$U = ku_C \tag{4}$$

where u_A, u_B, and u_C are uncertainty types A, B, and C, respectively; x is the brightness of the silk; A is the repeatability error of instrument measurement; k is the coverage factor; and U is the extended uncertainty.

According to equations (1)–(4) and Table 1, as a result, u_A is 0.06, u_B is 0.06, u_C is 0.08, and U is 0.14.

The total color difference can be calculated according to equation (5)[31,50]

$$\Delta E* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \tag{5}$$

where L^* indicates light and dark ($+\Delta L^*$, bright; $-\Delta L^*$, dark); a^* indicates red and green ($+\Delta a^*$, redder; $-\Delta a^*$, greener); b^* is yellowish blue ($+\Delta b^*$, yellower; $-\Delta b^*$, bluer).

The surface morphology of the silk fabrics was observed by SEM (Quanta250*, USA) with a voltage of 12.5 kV at 5000× magnification.

FTIR measurements were performed using a Thermo Nicolet Omnic sampler (Nicolet 5700, Thermo Nicolet Instrument Co., Ltd, USA). A total of 64 scans were taken for each sample between 650 and 4000 cm^{-1} with a resolution of 8 cm^{-1}. Three samples were used. The average spectra for the fibers of various chemical treatments were used for the evaluation.

Results and discussion

TGase enzyme reaction mechanism

TGase is an enzyme that can catalyze the reaction of amido groups, which crosslinks protein by an acyl group between the ε-amino group on the protein lysine and γ-amido group on glutamic acid, resulting in covalent crosslinking between proteins (or polypeptides) to form the corresponding polymerization product.[51] Due to the lack of glutamine residue in silk, it is necessary to add the SC for the formation of biopolymers. The reaction formula[40] is shown in Figure 1.

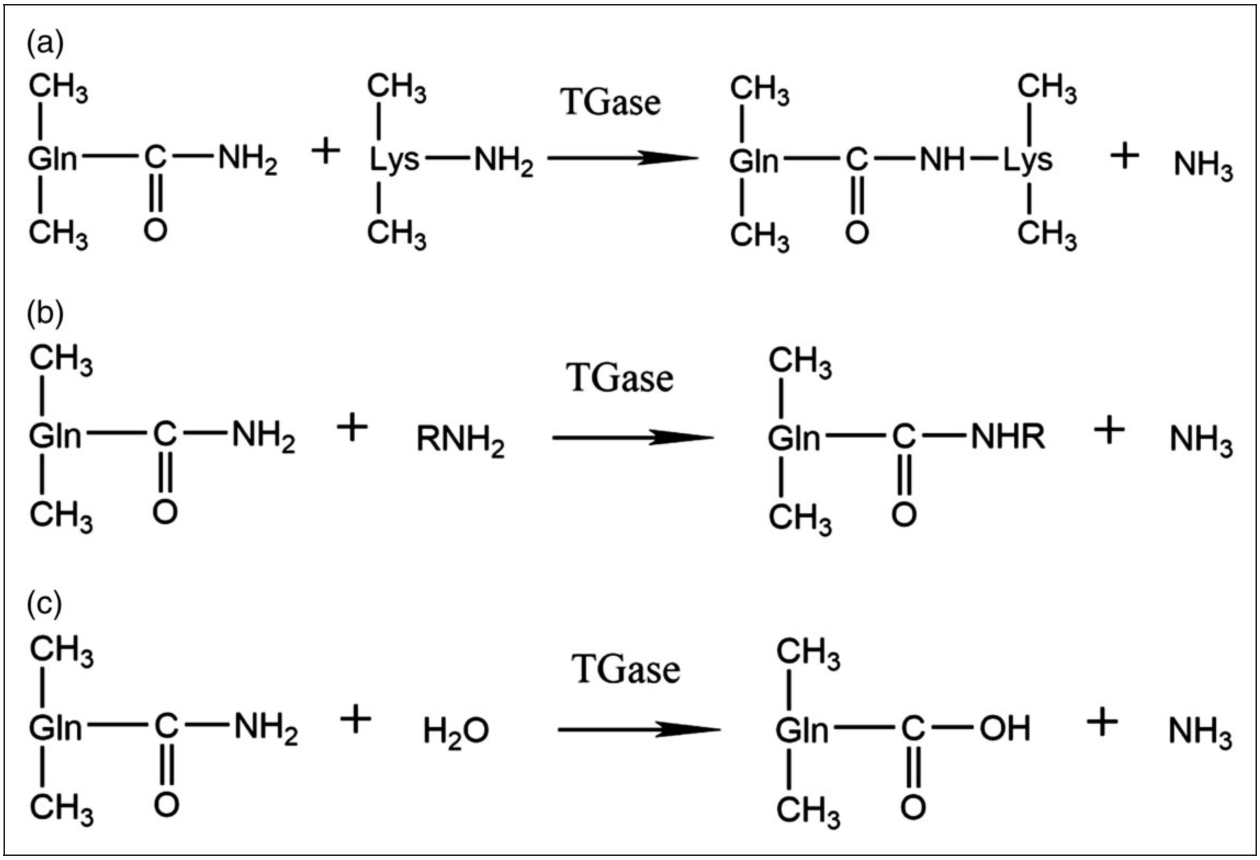

Figure 1. Transglutaminase (TGase) enzyme reaction: (a) lateral binding reaction; (b) acyl transfer reaction; (c) deamination reaction.

Under the catalysis of TGase, SC reacts with the primary amino acid (RNH_2) and lysine (Lys-NH_2) on the protein molecules on the silk fabric to form a crosslinked structure, and then sprays a transparent SF film for an enhanced crosslinking structure. A schematic diagram of the mechanism of TGase catalyzing the crosslinking of SC on silk fabric is shown in Figure 2.

Optimization of SC parameters catalyzed by TGase

The optimized process parameters are more convenient for subsequent experiments. The reaction temperature, time, TGase, and SC contents are selected as the four variables of the reinforcing silk fabric, and the breaking stress and strain are used as the evaluation for parameter optimization.

The breaking stress and strain of TGase and SC treated silk fabrics increase significantly with temperature, and the stress and strain at the breakage point decrease significantly at around 50°C, as shown in Figure 3(a). This is because the activity temperature of the TGase enzyme is at 45–50°C.[39,40,52] The higher the activity is, the more the crosslinking of the catalytic reaction there is. Therefore, the following experiment can be carried out at 50°C as an optimum temperature. As indicated in Figure 3(b), when the reaction time is 2 hours, the fracture stress and strain of the fabric decrease significantly. The results demonstrate that the longer time is in favor of the formation of a crosslinked structure until the reaction of the enzyme reaches saturation. Therefore, the reaction time is determined to be 2 hours. Similarly, according to Figures 3(c) and (d), the concentrations of TGase and SC were set to 1.5 mL and 2%, respectively.

After the optimal process parameters are determined, the experiment is repeated according to the optimized process, and the stress–strain curve is shown in Figure 4. The breaking stress and strain of TGase and SC reinforcement samples are significantly higher than that of the raw silk fabric. The breaking stress and strain of the silk fabric are increased by 17.08% and 38.61%, respectively, compared with the untreated fabric. Under the small deformation of force, the initial modulus of the reinforced silk fabric is lower than that of the original silk fabric, indicating that the ability of silk fabric resists the small deformation to increase. For comparison, when there is no TGase, the breaking stress and strain of the SC treatment sample are only increased by 10% and 9%, respectively, due to the

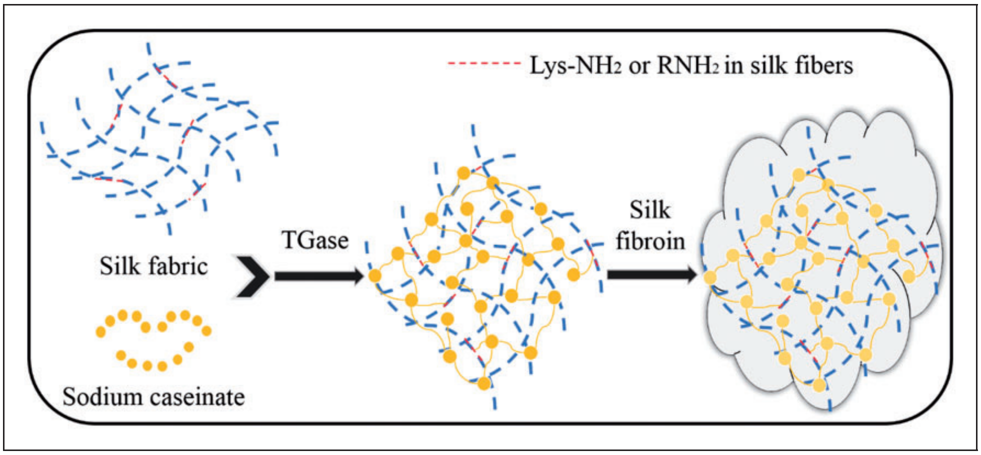

Figure 2. Schematic diagram of the sample preparation process. TGase: transglutaminase.

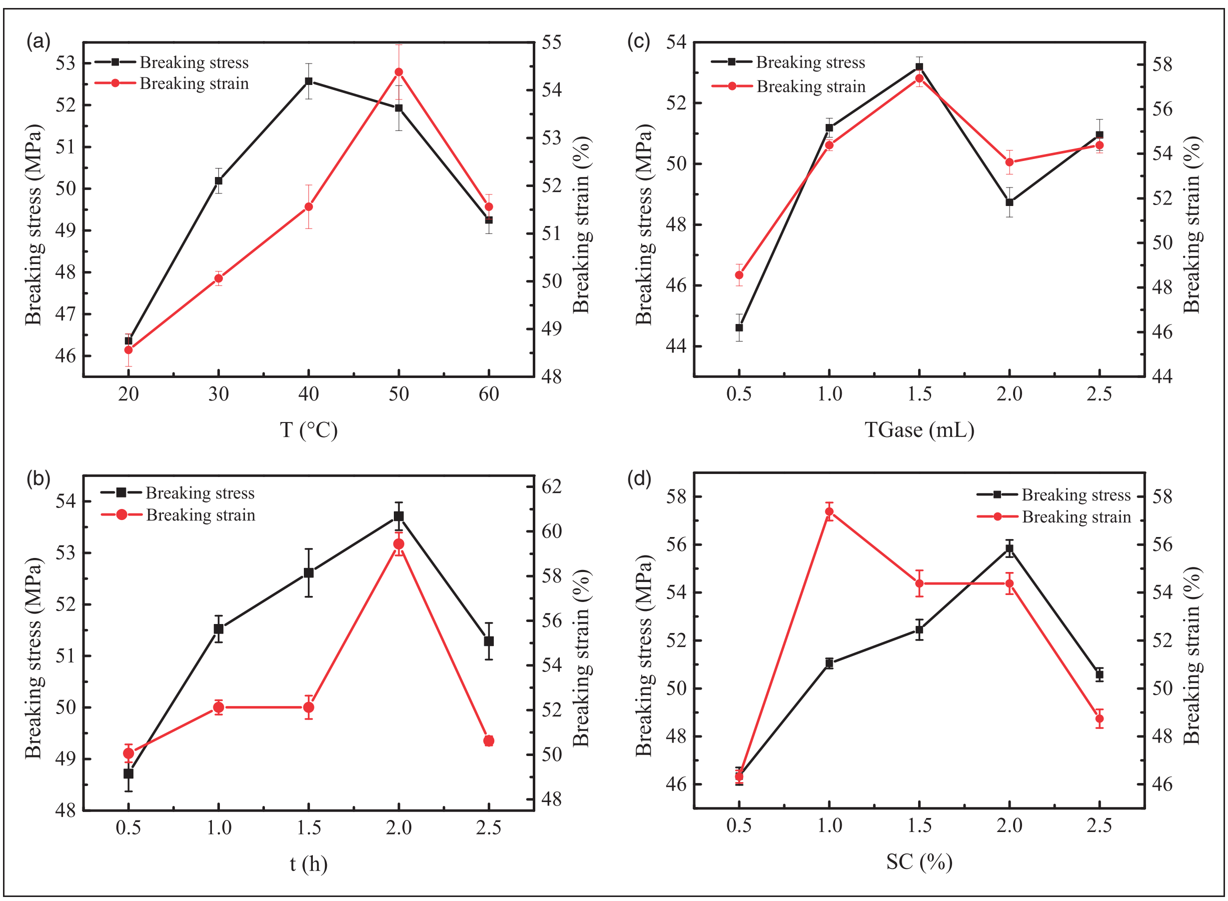

Figure 3. The changes of breaking stress and strain of transglutaminase (TGase) + sodium caseinate (SC) samples with (a) temperature *T* (°C), (b) time *t* (h), (c) TGase (mL), and (d) SC (%).

decrease of covalent bonds. The results indicated that the TGase enzyme catalyzes the crosslinking of protein molecules in SC and silk fabrics, and the formed network structure can resist the tensile deformation during the stretching process.

The untreated fabric and the reinforced fabric were simultaneously subjected to a washing experiment and washed for 10 cycles. The tensile strength of the washed fabric was tested and the stress–strain curve is shown in Figure 4. We can see that the breaking stress and strain

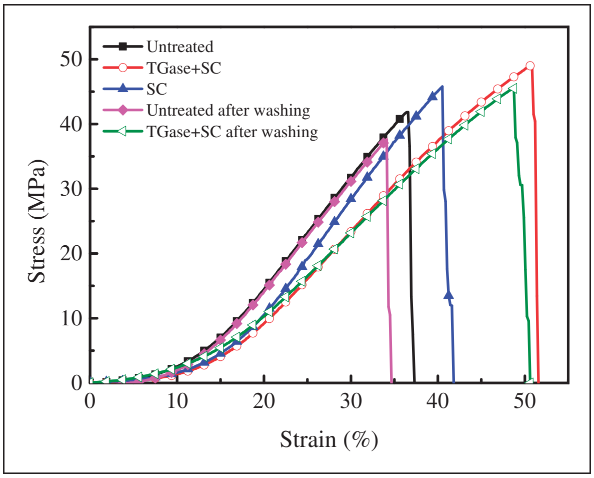

Figure 4. Under the optimal conditions (50°C, 2 h, 1.5 mL transglutaminase (TGase), 2% sodium caseinate (SC)), the stress–strain curve of the fabric.

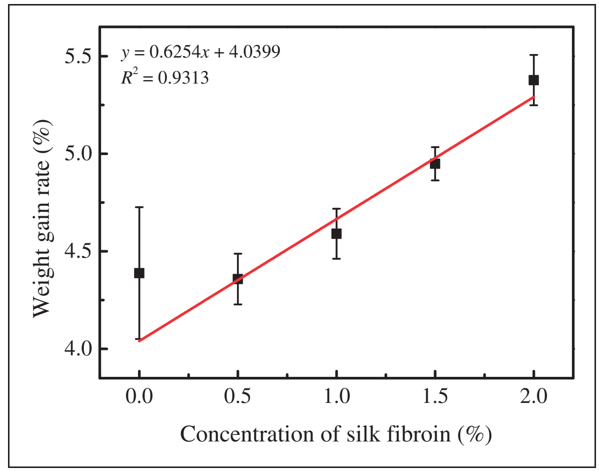

Figure 5. The weight gain rate of fabrics with the concentration of silk fibroin.

of the untreated and reinforced samples are slightly decreased by 6.91% and 8.8%, respectively. This shows that the reinforcing silk fabric has good washing durability.

Weight gain rate of the fabrics

In order to further reinforce the above treated silk fabric, the various concentrations (0, 0.5, 1.0, 1.5 and 2.0%) SF was sprayed on the treated silk fabric. For the sake of characterization of the attachment of reinforced materials to the fabric, the weight of treated fabric was tested and the weight gain rate was calculated as shown in Figure 5. The weight gain rate without SF was 4.38% compared to the raw silk fabric, indicating that the formation of biopolymer due to the crosslinking of TGase and SC. After spraying SF, the weight gain rate increases as the SF concentration increases with a good linear correlation, and the correlation coefficient is 0.9313, as shown in Figure 5. When the concentration of silk fibroin solution is 0.5%, the weight gain rate is less than 0% SF in Figure 5. This phenomenon can be explained by the fact that there are few SF crosslinked structures formed. Meanwhile, a large amount of water causes less stable macromolecular polymer produced by the enzymatic reaction, resulting in a slight decrease in the increase rate of weight.

Mechanical propertied of the fabrics

The mechanical properties of the treated silk fabric are characterized by the stress and strain curves, as shown as Figure 6(a). The breaking stress and strain of TGase-catalyzed SC strength silk fabrics were significantly higher than those of the untreated samples due to the crosslinking between protein molecules. When SF was sprayed on the surface of silk fabrics treated by TGase and SC, the breaking stress increases first and then decreases with increasing SF concentration, reaching an extreme value of 50.25 MPa at 1%. Meanwhile, the breaking strain decreased, indicating that SF is bonded to the silk fabric by hydrogen bonding and reinforced, as shown in Figure 6(b). The crosslinking structure produced by TGase-catalyzed SC and the combination of SF with silk fabric make the silk fabric consolidate into a new structural network and enhance the mechanical properties. Furthermore, when the SF concentration is 1%, the breaking stress and strain of the treated silk fabric were increased by 20.89% and 27.15%, respectively, compared to those of the untreated fabrics, according to Figure 6(b).

In order to compare the relationship between the weight increase rate and the mechanical properties, the changes of breaking stress and strain of the fabric with the weight gain rate are plotted as shown in Figure 7. It can be seen from Figure 7 that the breaking stress of the fabric increases and then decreases with the increase of the weight gain rate. The breaking strain is generally reduced. When the weight gain rate is below 4.59%, the breaking stress increases, which is attributed to the consolidation of the macromolecules. However, the breaking stress reduces when the weight gain rate is above 4.59%. This is because the greater the weight of the fabric, the more SF is filled in the pores of the fabric, which limits the free slippage of the fibers. When the fabric is stretched by an external force, the material filled in the pores of the fabric hinders elongation of the fiber, resulting in breakage of the fiber. This also explains the phenomenon that the breaking strain decreases with the fabric weight gain rate increases. Meanwhile, evaluating the effect of the weight gain

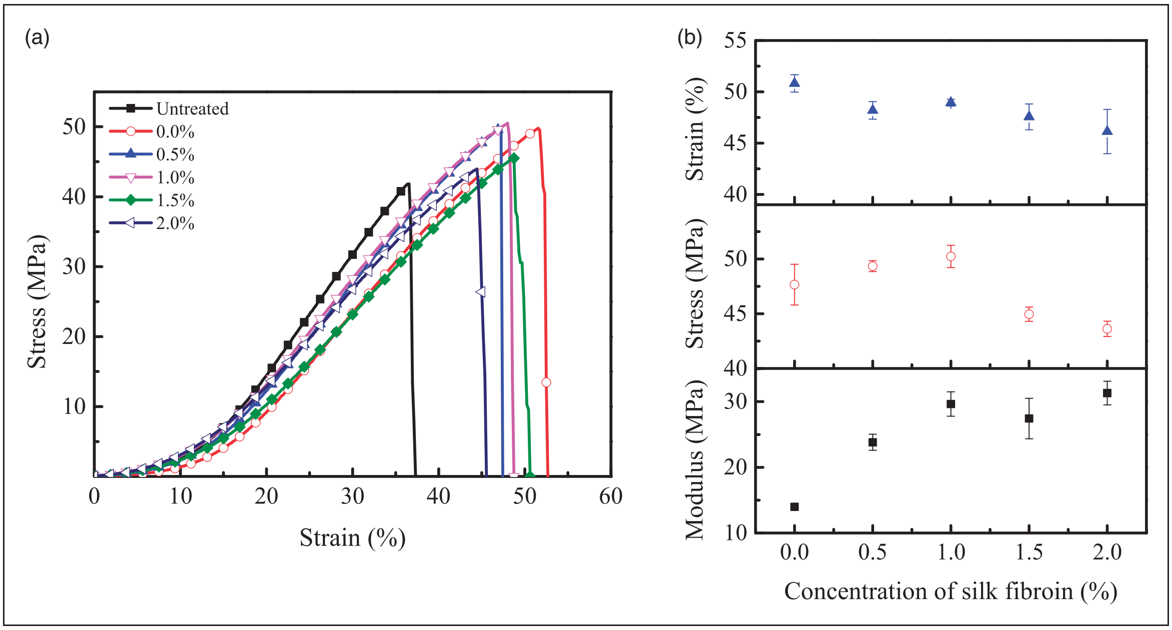

Figure 6. (a) Stress–strain curves and (b) mechanical properties of the silk fabrics treated by transglutaminase + sodium caseinate and sprayed with silk fibroin with different concentrations.

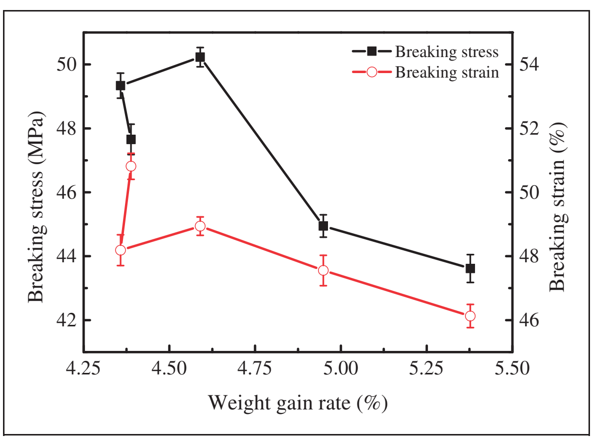

Figure 7. Breaking stress and strain change with weight gain rate of the fabrics.

rate of the fabric on the fracture stress and strain is effective for controlling the amount of reinforcing material in the protection of silk fabrics.

Color difference analysis of the fabrics

The color difference should be reduced as much as possible in the protection of textile artifacts and is tested between the untreated and treated samples. According to Table 2, the total color difference (ΔE^*) increases as the SF concentration increases. When the SF concentration is 1%, ΔE^* is 0.99, which satisfies the acceptable range of color difference in the field of conservation of cultural heritage (<1.00).[19] Meanwhile, there was no significant change in the color of the treated fabric photos, indicating that this method does not affect the visual effect.

Table 2. L*a*b* values of the silk fabrics treated by transglutaminase + sodium caseinate and sprayed silk fibroin (SF) with different concentrations

Concentration of SF (%)	ΔL^*	Δa^*	Δb^*	ΔE^*
Untreated fabric	0	0	0	0
0.0	−0.17	−0.30	0.83	0.90
0.5	−0.42	−0.30	0.70	0.86
1.0	−0.56	−0.29	0.76	0.99
1.5	−0.67	−0.31	0.78	1.07
2.0	−0.73	−0.32	0.85	1.17

Images of the treated silk fabric under irradiation of UV light from a fluorescence microscope are shown in Figure 8. It can be observed from the photograph of the fabric that the brightness the SF treated increases fabric compared with that of the untreated fabric, possibly because the fabric forms a film covering the fabric after the SF solution treatment, which reduces a large amount of diffuse reflection. The gloss of the fabric does not appear to be uniform and soft compared to the untreated fabric, but gradually gives a strong luster. In addition, a warp yarn is peeled from the fabric, and the picture under the irradiation of UV light is shown in Figure 8. The untreated single yarn looks loose and has

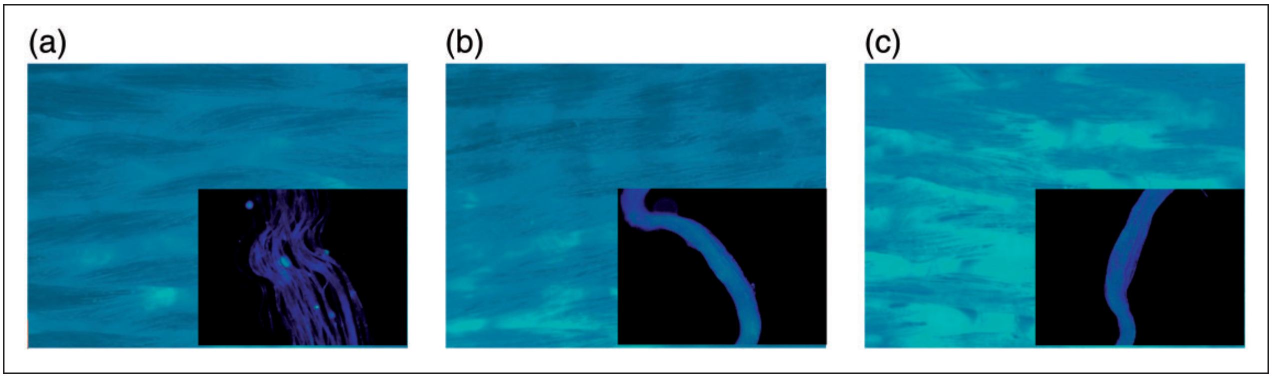

Figure 8. Photograph of silk fabric under the ultraviolet light of a fluorescence microscope: (a) untreated; (b) transglutaminase (TGase) + sodium caseinate (SC); (c) TGase + SC + 1.0% silk fibroin.

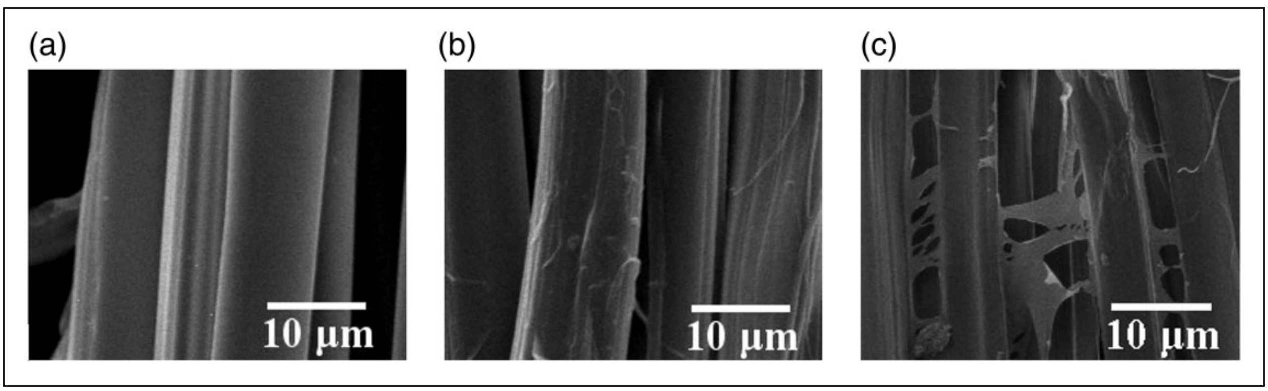

Figure 9. Scanning electron microscopy images of silk fabric: (a) untreated; (b) transglutaminase (TGase) + sodium caseinate (SC) treated; (c) TG + SC + silk fibroin treated.

a blue light spot, which is the fluorescence phenomenon of the silk fiber itself. After the reinforcement treatment, it can be observed that the fibers in the yarn are bonded and clasped, indicating that the silk fiber is reinforced.

SEM analysis of the fabrics

The surface morphology analysis of the silk fabric is shown in Figure 9. The surface of the untreated silk fabric is smooth and flat, as illustrated in Figure 9(a). When the silk fabric is treated with the optimal parameters of TGase and SC, the surface of the fabric is rougher than that of the untreated sample, and there is a layer of adhesive on the surface of the fiber, as indicated in Figure 9(b), which can be considered as a biomacromolecule polymer produced by TGase-catalyzed SC. When a layer of 1% SF solution is sprayed on the enzymatically reinforced sample, it forms a film on the surface of the silk fabric by the hydrogen, salt, and ester bond, as shown in Figure 9(c).

FTIR analysis of the fabrics

FTIR analysis is one of the analytical methods for the secondary structure transformation of silk fabrics. There was no new peak formed for the FTIR spectra of the treated silk fabric, as shown in Figure 10(a), indicating that the reinforcing material used is the same as the silk fabric and has good compatibility. The Gaussian curve of the amide I band region (1720–1573 cm^{-1}) in the infrared spectrum of the silk fabric was fitted into 11 peaks centered around 1611, 1619, 1624, 1630, 1640, 1650, 1659, 1666, 1680, 1691, and 1698 cm^{-1}, according to the literature.[53] The area percentage change in the peak of the four types of secondary structures (β-sheet, α-helix, random coil, and β-turn) in the silk fabric is shown in Figure 10(b). When the concentration of SF is 1%, the β-sheet structure in the treated fabric increases 8.96% compared with that of the untreated sample, confirming that crosslinked structures are formed in the silk fabric. The increase of the β-sheet structure will provide a more compact structure and a higher

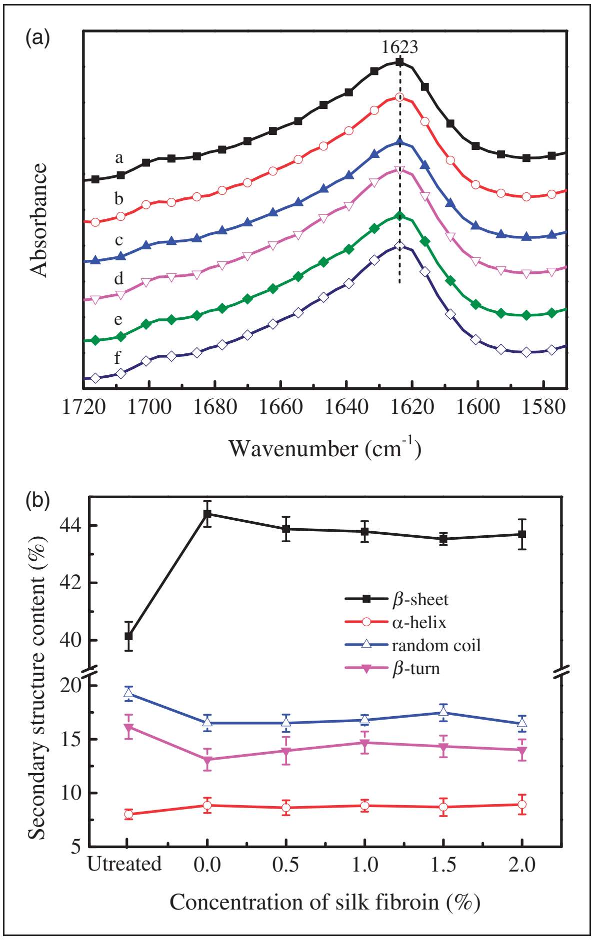

Figure 10. (a) Fourier transform infrared spectra and (b) the fitting results of the secondary structure of silk fibers treated with different concentrations of silk fibroin (SF). (Samples. a: untreated; b: transglutaminase (TGase) + sodium caseinate (SC); c: 0.5% SF + TG + SC; d: 1% SF + TG + SC; e: 1.5% SF + TG + SC; f: 2% SF + TG + SC.)

resistance to chemical attack. Due to the presence of small molecules, the spraying of the SF solution causes a slight decrease in the content of the β-sheet structure with the increase of concentration of SF, but it is still much higher than that of the untreated sample. The increase of the α-helix structure after TGase and SC treatment is due to crosslinking between molecules. The homology and affinity of the SF solution to the silk fabric can form hydrogen bonds, salt bonds, and ester bonds with the reactive groups on the silk fibers.[21,23] Therefore, the α-helix structure exhibits a slight increase, and the rigidity of the hydrogen bond leads to an increase in bending stiffness. The random coil structure is crosslinked into a more stable β-sheet, exhibiting a reduced tendency. As a result, the movement of the molecules is limited, leading to an increase in the rigidity of the fabric. β-turn contains the donor glutamine residue required for the catalytic reaction, so it is significantly reduced after reinforcement. The results of infrared spectral curve fitting analysis are consistent with the analysis of stress and strain (Figure 6) of treated fabrics.

The protection of artificially aged silk fabrics

Mechanical properties analysis of the fabrics. Due to the scarcity and preciousness of historical silk fabrics, researchers usually use artificial aging methods to simulate the aging of historical silk fabrics, and alkali hydrolysis aging[21,23] is one of them. In order to verify the application effect of the protection method proposed in this paper on historical silk fabrics, the method was applied to the alkali-hydrolyzed aged silk fabric. Among the many corrosive substances, acid and alkali have the strongest stagnation for silk fiber, and continuous alkaline hydrolysis aging accelerates the degradation of SF molecules between the polypeptides, resulting in the decrease of crystallinity and mechanical properties. The stress–strain curves of the fabrics are shown in Figure 11(a). To further analyze the mechanical properties of the fabric, the changes in breaking stress and strain, initial modulus, and fracture work are discussed, as shown in Figure 11(b). Compared to untreated fabric, the breaking stress and strain of the silk fabric decreased by 92.28% and 19.47% after alkali aging, respectively. The initial modulus and fracture work of the alkali aging sample decreased by 86.2% and 95.54%, respectively, indicating that the protein macromolecular chain in the silk fabric was broken by the action of the alkali, resulting in the mechanical properties being destroyed and decreasing. The surface morphology of the alkali aging sample is hydrolyzed into debris according to Figure 12(a), leading to the damage of mechanical properties. The breaking stress and strain of the reinforced sample increased by 37.77% and 13.38%, respectively, relative to the aged sample. In conclusion, the method of the cross-linked silk fabric by the TGase enzyme and SF solution has good protection for the reinforcement of alkali-hydrolyzed aging silk fabric. The initial modulus of the alkali aging sample did not change significantly after the reinforcement, but the fracture work increased significantly from 164.84 to 285.53 J, an increase of 73.22%, indicating that the toughness and intrinsic binding energy of the reinforced fabric is significantly improved. This phenomenon is attributed to the formation of reticulated macromolecular polymer by the TGase enzyme, which catalyzes the SC in the pores of the silk fabric. Crosslinking of the SF solution also leads to an increase in the toughness of the fabric.

SEM analysis of the fabrics. SEM images of the alkali-hydrolyzed aged silk fabric and reinforced sample are

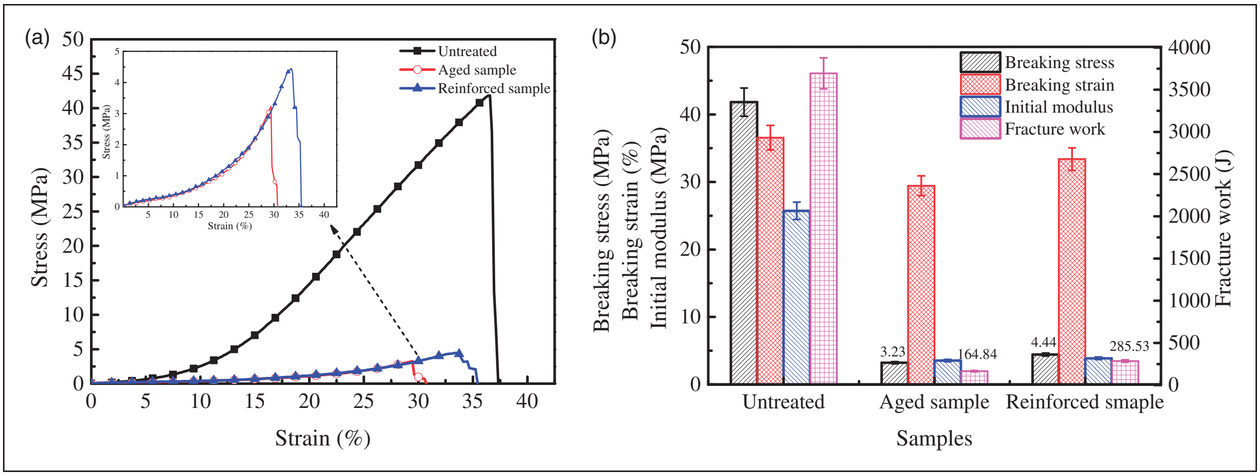

Figure 11. Alkaline aging and reinforcement samples: (a) stress–strain curve; (b) mechanical properties.

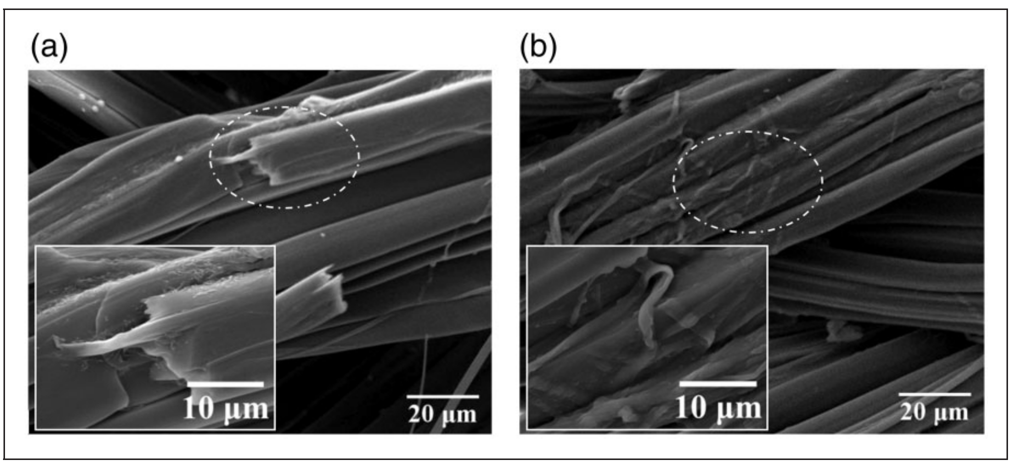

Figure 12. Scanning electron microscopy images: (a) alkali aging sample; (b) alkali aging reinforcement sample.

shown in Figure 12. According to Figure 12(a), the surface of the silk fabric has more longitudinal grooves, accompanied by fiber breakage, and the fibers are hydrolyzed into pieces and attached to the fiber, even leading to the breakage of silk fiber. This is because silk fabric protein molecules are very susceptible to corrosion by alkali. When the aged sample is reinforced, the surface of the silk fabric that had the longitudinal grooves is repaired by the reinforcing material, which is well adhered to the silk fiber. In this way, the mechanical properties of the silk fabric are improved, and the double crosslinking of the enzymatically produced macromolecular polymer and the SF molecule protection plays an important role.

FTIR analysis of the fabrics. Vilaplana et al.[16] believe that alkaline hydrolysis aging does not seem to affect tyrosine, but rather it leads to the hydrolysis of amorphous regions and amino acids with bulky side groups, which seems to be the weakest hydrolysis point in silk structures. Therefore, alkaline hydrolysis aging has a greater impact on the secondary structure of the silk fabric. Figure 13(a) shows an infrared spectrum of the amide I band of the silk fabrics. In order to analyze the internal structure of the fabric, a Gaussian fitting process is performed on the amide I band to calculate the percentage of each fitting peak. The content of the secondary structure of the silk fabric is as shown in Figure 13(b). The contents of the β-sheet structure and the α-helix in the silk fabric after alkali hydrolysis aging are reduced by 5.06% and 46.88%, respectively, indicating macromolecule cleavage in the amorphous region by alkali. The β-sheet and the α-helix conformation of the reinforced sample are increased by 16.82% and 0.93%, respectively, compared with the aged sample, and the random coli and β-turn conformation were reduced by

17.76% and 22.55%, respectively. The method has a good reinforcing effect on alkali-hydrolyzed aged silk fabric.

Life prediction of the silk fabrics. Sebera[54] proposed that the PI was a relevant tool for quantifying the effects of environmental temperature (T) and relative humidity ($RH\%$) on the useful life expectancy of paper-based collections. According to Sharif and Esmaeili,[55] Krüger and Diniz,[56] and Gray,[57] based on the influence of environment temperature and humidity on the long-term preservation of the fabric, the PI is used to predict the longevity of the treated silk fabric. The treated samples (TG + SC + 1% SF) are transferred from a temperature of 25°C and a humidity of 65% to an oven at 125°C and 135°C for 24 hours, respectively. The breaking stress at different temperatures is shown in Table 3. The activation energy ΔH^+ (kJ/mol) of the fabric is calculated according to Equation (6), and the values ΔH^+ of treatment are 10.47, 16.89, 12.79 kJ/mol at 135°C, 160°C, 180°C, respectively. The PI can be calculated according to Equation (7). The breaking stress loss at 125°C ($RH\% = 22\%$) is chosen as a reference and can be calculated at 135°C ($RH\% = 18\%$) with a PI value of 0.96 years, that is, 350 days. Similarly, the PI values at 160°C ($RH\% = 9\%$) and 180°C ($RH\% = 7.2\%$) are 0.62 years (226 days) and 0.30 years (110 days), respectively, indicating that higher temperature and humidity are not conducive to the preservation of silk fabric. This is because thermal oxidation and thermal cracking of silk fibers at higher temperatures cause the bond between macromolecular chains to be destroyed, and moisture plays an important role in various chemical reactions.[4,6] The same method is used to calculate the PI of untreated samples at different temperatures. The PI values of untreated samples at 135°C, 160°C, and 180°C are 0.80 years (292 days), 0.56 years (204 days), and 0.26 years (95 days), respectively. Compared with the untreated sample, the storage index of the reinforced fabric at 135°C, 160°C, and 180°C increased by 20%, 10.71%, and 15.38%, respectively, indicating that the reinforced silk fabric has better aging resistance.

We can apply the PI calculation method to predict the storage time under different temperature and humidity conditions and evaluate the lifetime of silk fabrics. For example, when the temperature is 160°C,

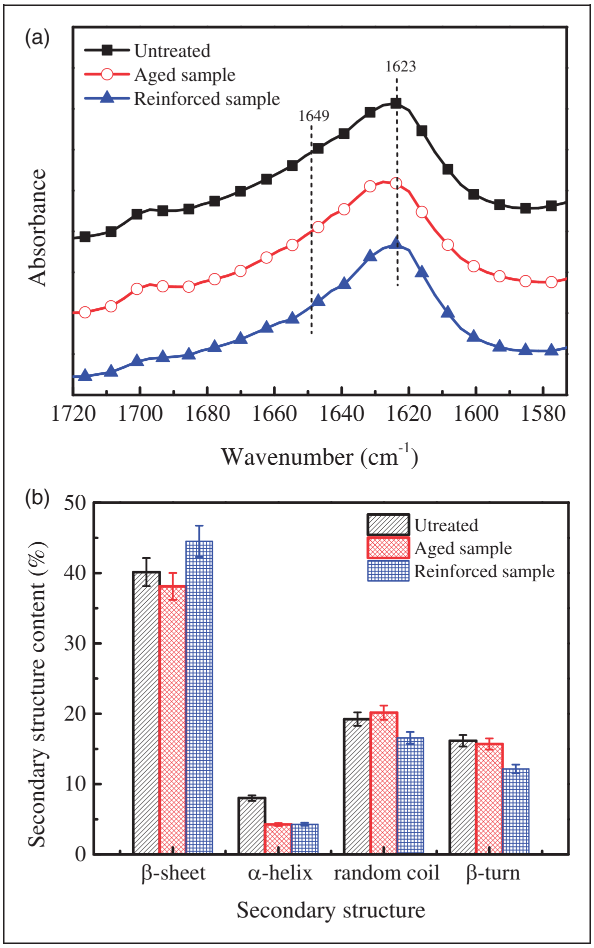

Figure 13. Alkaline aging and reinforcement samples: (a) infrared spectrum; (b) secondary structure content.

Table 3. Breaking stress loss after aging and the preservation index (PI) at different temperatures of silk fabrics

		Breaking stress (MPa)		Breaking stress loss (MPa)		PI (years)	
	Temperature (°C)	Untreated	TG + SC + SF	Untreated	TG + SC + SF	Untreated	TG + SC + SF
T0	25	47.69 ± 1.32	48.20 ± 2.12	–	–	–	–
T1	125	42.27 ± 2.51	42.32 ± 3.17	5.42 ± 1.92	5.88 ± 2.65	–	–
T2	135	38.41 ± 2.27	40.07 ± 1.64	9.28 ± 1.80	8.13 ± 1.88	0.80	0.96
T3	160	13.43 ± 1.36	15.20 ± 3.16	34.26 ± 1.34	33.00 ± 2.64	0.56	0.62
T4	180	6.39 ± 2.71	8.17 ± 2.22	41.3 ± 2.02	40.03 ± 2.17	0.26	0.30

TG: transglutaminase; SC: sodium caseinate; SF: silk fibroin.

Table 4. Breaking stress loss after aging and preservation index (PI) at different temperatures of alkali aged fabric

	Temperature (°C)	Breaking stress (MPa)		Breaking stress loss (MPa)		PI (years)	
		Untreated	TG + SC + SF	Untreated	TG + SC + SF	Untreated	TG + SC + SF
T0	25	3.23 ± 0.12	4.44 ± 0.38	–	–	–	–
T1	125	3.12 ± 0.32	4.35 ± 0.42	0.11 ± 0.23	0.09 ± 0.4	–	–
T2	135	2.88 ± 0.25	4.26 ± 0.15	0.35 ± 0.19	0.18 ± 0.27	0.48	0.70
T3	160	0.91 ± 0.05	2.99 ± 0.08	2.32 ± 0.09	1.45 ± 0.23	0.20	0.25
T4	180	0.02 ± 0.02	2.13 ± 0.06	3.21 ± 0.07	2.31 ± 0.22	0.19	0.22

the humidity is increased from 9% to 45%, and then the PI value becomes 0.11 years (40 days). Conversely, when the humidity is 9%, the temperature is lowered from 160°C to 25°C, and then the PI is 0.47 years (172 days). In summary, it is possible to predict the longer-lasting life by changing the temperature for exploring the environmental conditions that protect the cultural relics

$$\Delta H^{+} = \frac{-2.303(1.9872)(\log \frac{k_1}{k_2})}{1/(T_1 + 273.15) - 1/(T_2 + 273.15)} \quad (6)$$

where k_1 is the breaking stress loss (MPa) at T_1 (°C) and k_2 is the breaking stress loss (MPa) at T_2 (°C)

$$PI = \frac{P_2}{P_1} = \frac{RH_1}{RH_2}\left(\frac{T_1 - 460}{T_2 - 460}\right)10^{394\Delta H^{+}\left(\frac{1}{T_2+460} - \frac{1}{T_1+460}\right)} \quad (7)$$

where P_1 and P_2 are the permanence for the (T_1, RH_1) and (T_2, RH_2) conditions, respectively. PI is the permanence of P_2 relative to P_1.

The same calculation method was applied to the alkali aging samples and their reinforcement samples. The results are shown in Table 4. It can be seen that the PI values of the reinforcement sample at 135°C, 160°C, and 180°C are 0.70, 0.25, and 0.22, respectively. The PI values of the alkali aging sample at 135°C, 160°C, and 180°C are 0.48, 0.20, and 0.19, respectively, from which can be obtained that the reinforced silk fabric has better aging resistance.

Conclusions

This work has demonstrated that the SF solution is sprayed on TGase and SC treated samples to effectively protect and strengthen silk fabrics. The selected materials are safe and harmless, which have good biocompatibility with silk fabrics because of the protein composition of TGase and SC. The polymer produced by the reaction of TGase and SC proved to have good stability after washing. Compared with the untreated fabric, the breaking stress and strain of the reinforced silk fabric are increased by 20.89% and 27.15%, respectively, when the SF concentration is 1.0%. Moreover, the color difference results indicate that this method does not affect the aesthetic appearance of the silk fabric. In addition, the increase of the β-sheet structure will provide a more compact structure and a higher resistance to chemical attack, which improves the mechanical properties of silk fabric. In order to verify the application of this method in the protection of ancient silk fabrics, the artificial accelerated alkali hydrolysis aging silk fabric is protected by this method, indicating that the method has a good recovery and enhancement effect on the aged samples. The PI is proposed to evaluate for the lifetime of the reinforced silk fabric by the change of temperature and humidity, illustrating that higher temperature and humidity lead to a lower PI, indicating that the reinforced silk fabric has better aging resistance. This method is expected to be applied to the protection and restoration of historical silks.

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