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Fibroin–EGDE Consolidation: A New Method for Conserving Fragile Silk Textiles

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(biographies and contact information for all authors can be found at the end of this paper)

Abstract

Many consolidation methods have been used to conserve fragile silk textiles. However, there is still much room for improvement. This paper reports on a new consolidation method for fragile silk conservation, which features a combination of fibroin and ethylene glycol diglycidyl ether (EGDE). We chose the fibroin protein as the consolidant because fibroin is one of the main silk proteins and is the structural centre of the silk. The consolidation process involved spraying the fibroin-EGDE solution onto the silk surface to form an even veneer in ambient temperature and humidity. Adhesive veneers were built up in multiple layers, with each layer drying before the next step. After optimizing solution concentrations, activation temperature, time, solvent, and pressure, we chose a standard protocol to apply the fibroin-EGDE solution onto fragile silk textiles. We then analysed some physical properties (such as the breaking strength, elongation at break, chromatic aberration, and flexibility) of the consolidation samples. The samples were also analysed by Fourier transform infrared spectrophotometry, ¹³C nuclear magnetic resonance spectra, amino acid analyser, X-ray diffractometry, thermogravimetry, and scanning electron microscopy. The results showed that the fibroin-EGDE method provides excellent consolidation of fragile silks. Finally, the fibroin-EGDE method was applied to consolidate a silk relic made in the Liao Dynasty (907–1125). Both the breaking tenacity and breaking elongation of the fragile silk were improved upon consolidation treatment, while the appearance of the silk remained visually unaltered.

Titre et Résumé

La consolidation à l'aide de fibroïne–EGDE : une nouvelle méthode pour traiter la soie dégradée

De nombreuses méthodes de consolidation ont été employées pour restaurer la soie dégradée, mais bien des progrès peuvent encore être réalisés en ce domaine. Le présent article porte sur une nouvelle méthode de consolidation de la soie dégradée, dont l'élément clé est la combinaison de la fibroïne et du diglycidyléther d'éthylèneglycol (EGDE). Nous avons choisi la fibroïne comme agent de consolidation, car celle-ci constitue une des principales protéines de la soie et un constituant de base de sa structure. Le procédé de consolidation consiste à vaporiser une solution de fibroïne-EGDE à la surface du tissu de soie, dans des conditions correspondant à celles de l'humidité relative et de la température ambiantes, afin d'y former un revêtement uniforme. Le revêtement d'adhésif est produit sous forme de nombreuses couches successives, en s'assurant de laisser sécher la couche précédente avant d'en appliquer une nouvelle. Une fois l'optimisation de certains paramètres effectuée (les concentrations des solutions, la température d'activation, la période, la nature du solvant et la pression), nous avons élaboré une méthode normalisée d'application de la solution de fibroïne-EGDE sur des tissus de soie dégradée et fragile. Nous avons ensuite déterminé certaines propriétés physiques des échantillons

consolidés, notamment la résistance à la rupture, l'allongement à la rupture, l'aberration chromatique et la flexibilité. Les échantillons ont aussi été analysés en utilisant diverses techniques, dont la spectrophotométrie infrarouge à transformée de Fourier, la spectroscopie de résonance magnétique nucléaire du ^{13}C , la diffraction des rayons X, la thermogravimétrie, la microscopie électronique à balayage et l'analyse des acides aminés. Les résultats indiquent que la méthode de consolidation à l'aide de fibroïne-EGDE donne d'excellents résultats pour la soie dégradée. Finalement, cette méthode de consolidation a été employée pour traiter une relique en soie datant de la dynastie Liao (907 à 1125). Le traitement de consolidation a permis d'améliorer la résistance à la rupture et l'allongement à la rupture de la soie dégradée, sans toutefois en altérer l'aspect esthétique.

Introduction

The fragility of certain deteriorated fibers and textiles has caused particular conservation problems, and a wide variety of consolidation methods have been used over the centuries to enhance the long-term preservation of fragile silks. Consolidants can be applied directly to the silk as liquids and integrate into the silk fibers. At present, the widely-used consolidants include paraffin, cellulose nitrate, various gums, starches, natural or artificial resin, Parylene C, and graft copolymerization (Keyserlingk 1990; He 1994; Hu 1995; Zhang 1999). For the very fragile silk fabrics, consolidation is also a good choice for prolonging the life of the aged silk fabrics. Zhang and Yuan (2003) applied a new kind of silicone modified acrylic resin SA 6 to consolidated fragile silk fabrics. Their results suggest that SA 6 is good in anti-aging, anti-mold and stain-resistant properties. Hansen (1989) and Ginell (1989) studied the consolidation of fragile silk by Parylene-C, which was capable of providing some consolidating effect for weak fabrics. Masschelein-Kleiner and other authors studied the consolidating effect of treatment by several adhesives on silk (Heylen 1968; Steene 1980; Juliette 1984; Bergiers 1984; Masschelein-Kleiner 1986; Tsukada 2000). However, those methods have deficiencies in this way or another and certain restrictions limit their applications. Parylene C method adversely affects the characteristic of silk and the process is irreversible. Polymeric materials' aging products will affect silk relics' security.

To find the most appropriate adhesive and consolidant, therefore, became the main aim of this study. Adhesives and consolidants were sought, which were chemically inert and which did not cause deleterious reactions on the silk. The desirable quality was measured by evaluating the degree of changes in the appearance of the silk after consolidating and the stability of the adhesive and consolidant over time.

Silk fibroin, being biocompatible, non-toxic, non-polluting, non-irritating and biodegradable, shows obvious advantages over other natural or artificial polymers for the silk consolidation (Mori 2000). The reason that we chose fibroin to consolidate silk is because fibroin, as one of the major proteins in the silk, is homologous and compatible with fragile silk. A process for the chemical modification of silk fabrics by epoxy compounds has been developed in industrial scales in Japan (Tanaka 1969). EGDE is one of the most important modifying agents applied in industry. Researchers have analyzed the reactivity of epoxide with silk. Silk fibers and fabrics modified by epoxide showed improvements in the physical property, moisture absorption, chemical resistance, and wash and wear property (Shiozaki 1971; Tsukada 1993; Shiozaki 1994; Masanobu 1991).

To the best of our knowledge, there is no report on the consolidation of fragile silk fabrics with silk fibroin and EGDE. In this study, the modification effects were measured by using artificial aged silks, instead of silk relics. A fibroin-EGDE consolidation technique was applied to the consolidation of a silk relic made in the Liao Dynasty, an ancient Chinese dynasty spanning from the 10th to the 12th century (907-1125).

Experimental section

The silk fibroin was prepared in the following manner: fragile silk fabrics were prepared from white silk habutae fabrics, which were immersed in 50 g/L NaOH solution for 9 hours under 35°C and 50% relative humidity (RH). *Bombyx mori* silk fiber was degummed by treating twice with 5 g/L Na₂CO₃ solution at 98~100°C for 30 min each and air-dried. Deionized water was used throughout the study. Twenty grams (20 g) of the fibers was dissolved in 1000 ml 50% CaCl₂ solution at 96-98°C for 90 minutes. The solution was dialyzed in a cellulose tube (molecular cutoff of 14 kDa) against water for 3 days, and aqueous silk fibroin solution with impurity was obtained. Then the solution was put into a freeze drier. After 16 to 20 hours drying, pure silk fibroin powders were prepared and removed from the freeze drier. Finally, different silk fibroin solutions were prepared from the pure silk fibroin powders as experiment required.

The consolidation condition was chosen by varying factors including solution concentrations, activation temperatures and time. After a series of experiments, a standard process was decided for applying the fibroin-EGDE solution onto fragile silk fabrics. The fragile silk fabrics were first sprayed with 12.5 g/L silk fibroin solution in ambient temperature and humidity and subsequently with 50 g/L EGDE solution after 10 min. The samples were conditioned in air at 25°C for 2 days. To figure out the consolidation mechanism, four groups of experiments were carried out (Table 1).

Table 1 Sample preparations

Samples	12.5 g/L silk fibroin solution	50 g/L EGDE solution
FS	-	-
FS-F	+	-
FS-F-E	+	+
FE-E	-	+

Table 1 caption: FS— Fragile silk fabric; FS-F— Spraying 12.5 g/L silk fibroin solution on fragile silk fabrics; FS-F-E— Spraying 12.5 g/L silk fibroin solution on fragile silk fabrics until supersaturation, after 10 minutes spraying 50 g/L EGDE solution until supersaturation; FE-E— Spraying 50 g/L EGDE solution on fragile silk fabrics.

The consolidation efficacy was analyzed by testing both the physical properties, such as the breaking strength, elongation at break, color change and flexural stiffness and chemical properties of the consolidated samples. The breaking strength and elongation at break of the silk fabrics were measured with YG065 strength tester of fabric using the national standard technique (State Bureau of Technical Supervision, 1997) at 20°C and 65% RH at a gauge length

of 100 mm and strain rate of 100 mm/min. Each sample was tested for five times and the average value was calculated. The color change of the silk fabrics was measured with a SC-80C automatic colorimeter. Flexural stiffness of the silk fabrics was measured with a LLY-01 Electronic Instrument Stiffness. Chemical properties were measured by Fourier Transform infrared spectroscopy (FTIR) using a Nicolet 5700 FT-IR Spectrophotometer, Carbon 13 Nuclear Magnetic Resonance (^{13}C NMR) spectra (Recorded on a Bruker AMX300-WB working at 300.1 MHz on proton and 75.47 MHz on carbon with a 7-mm CP-MAS probe, under room temperature spinning at the magic angle (MAS) at a rate of 5 kHz.), amino acid analysis using a Hitachi L-8800 Type Rapid Amino Acid Analyzer, X-ray diffraction (XRD) using a ARL X' TRA diffractometer equipped with a $\text{CuK}\alpha$ tube, scanning electron microscope (SEM) with a JEOL JSM-5610LV scanning electron microscope at 15 kV acceleration voltage and thermogravimetric (TG) analysis running on a Pyris 1 instrument programmed under isothermal conditions, raised at $20^\circ\text{C}/\text{min}$ to 650°C .

Results and Discussion

Physical properties

Both the strength and elongation of the fragile silk fabrics increased dramatically upon the fibroin-EDGE treatment (FS vs. FS-F-E, Table 2). However, treatments with the fibroin protein (FS-F) or EGDE (FE-E) alone only slightly improved the strength and elongation of the fragile silk fabrics. A possible explanation is that EGDE facilitates the crosslinking between silk fabrics and silk fibroin molecules, as was supported by the evidence obtained from chemical property tests (shown later). The fibroin-EDGE-treated silk fabrics showed better handle than the original fragile silk fabrics, while the color of the silk fabrics was only slightly changed upon the treatment.

Table 2 Physical property of the silk fabrics

Samples	Strength(N)	Elongation(%)	Color change	Flexural stiffness (10^{-2}mN.m)
FS	1.54	2.00	/	1.08
FS-F	1.80	2.20	1.42	1.07
FS-F-E	15.80	8.00	0.67	0.71
FE-E	7.50	6.00	0.91	0.90

Chemical properties

Silk fabric samples with different treatments were analyzed in the range of 700 to 4000 cm^{-1} by FTIR spectra (Figure 1). Before FTIR test, the samples (FS-F-E) and (FE-E) were washed with deionized water to remove unreacted EGDE. The two peaks (858.3 and 931.6 cm^{-1}) that presented in the spectra of EGDE, which were representing the two ends cyclic ether characteristic absorption of EGDE, disappeared in the spectra of silk fabrics treated with fibroin-EGDE (FS-F-E) and EGDE (FE-E). Water-soluble unreacted EGDE was removed by washing (Figure 1B). A new peak at 1105 cm^{-1} , the characteristic absorption peak of EGDE representing the unsymmetrical stretching vibration of $-\text{CH}_2-\text{O}-\text{CH}_2-$ group, appeared in the

spectra of FS-F-E and FE-E treated samples. These results suggest that the silk fibroin molecules have been cross-linked by EGDE.

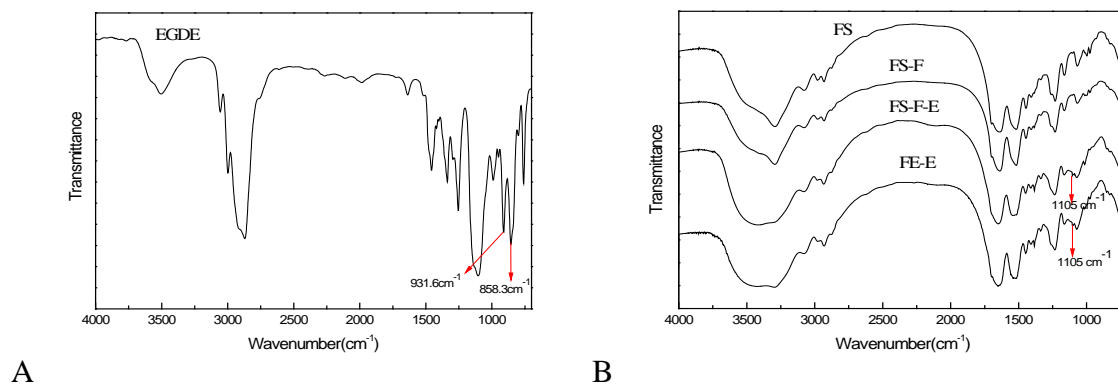


Figure 1. FTIR spectra of EGDE (A), FS , FS-F , FS-F-E and FE-E (B).

The major amino acids of silk fibroin are glycine, alanine, serine and tyrosine, so NMR spectra was mainly manifested in these amino acids. A new peak appeared at the 72.50 ppm in the ¹³C-CPMAS spectra of the FS-F-E group, which belongs to the middle of the alkyl ether in EGDE (Figure 2). This indicates that EGDE cross-links the group -CH₂-O-CH₂- of the silk fibroin. Moreover, one carbon atom of Tyr shifted from 157 ppm to 157.58 ppm in the FS-F-E sample, which might be a combination of chemical shifts of the tyrosine residue of the alkyl ether groups under shielding effect. Therefore, we believe that the cross-linking reactions occur between the tyrosine residues of fibroin and EGDE. Pro-nuclear role of the imidazole group of histidine and amino group of lysine are stronger than those on the hydroxylation of benzene, but their contents and the absorption rate in a magnetic field are too low to be detected in the NMR pattern.

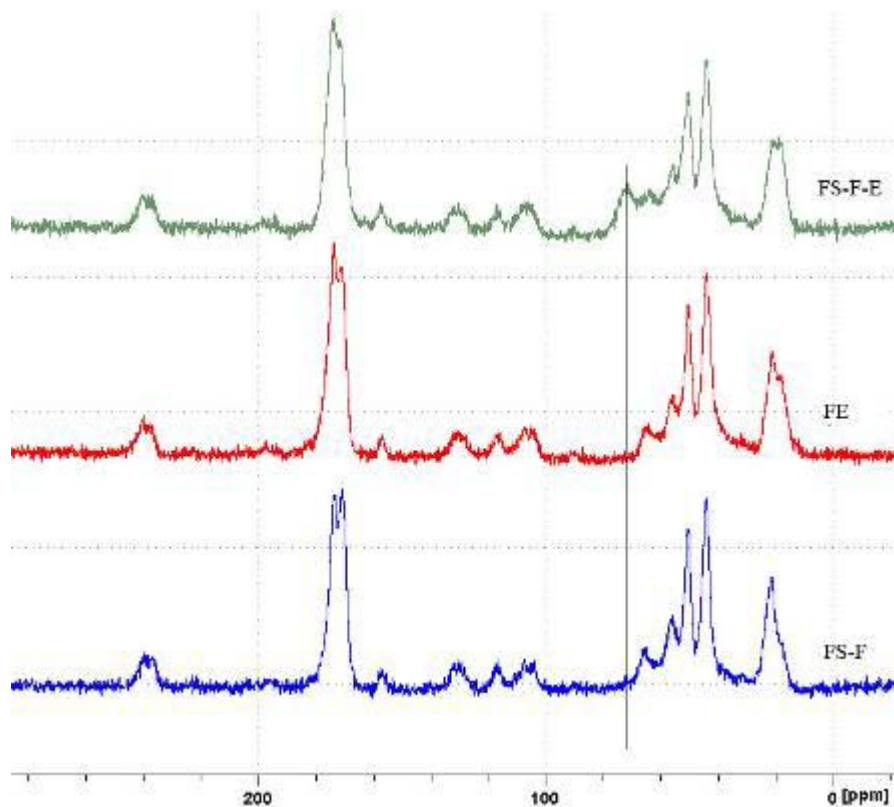


Figure 2. ^{13}C CP/MAS NMR spectra of FE, FS-F and FS-F-E. The chemical shifts are represented in ppm downfield from TMS.

The amino acid content of silk fabrics was analyzed to evaluate the reactivity of the functional amino acid residues of silk fibroin (Table 3). As reported previously, this analytical technique was useful for studying the formation of epoxide adducts by reaction with tyrosine and basic amino acid residues (lysine, histidine, and arginine) (Shiozaki 1969; Tanaka 1971). Other amino acids with functional groups reactive towards epoxides (aspartic and glutamic acids) should be reacted as well, but the covalent bond formed with epoxide molecule was easily broken during acid hydrolysis. The concentrations of most amino acids in FS-F-E increased, comparing those in the FS group. These changes may be due to the physical adsorption of the amino acids of fibroin on the surface of silk fabrics. However, the concentration of tyrosine in the FS-F-E group (9.58%) decreases from that in the FS group (11.51%). Among the basic amino acid residues, lysine and histidine decreased dramatically (Table 3). The changes of the composition of amino acids might result from chemical reactions between EGDE and the active groups of amino acids in tyrosine, lysine and histidine .

Table 3. Amino acid composition of silk fabrics.

Amino acids (mol%)	FS	FS-F-E
Glycine	37.14	37.36
Alanine	30.54	30.85
Valine	3.04	2.63
Leucine	0.08	0.51
Isoleucine	0.55	0.61
Serine	12.08	12.63
Threonine	0.79	1.05
Asparagine	1.21	1.44
Glutamine	0.86	1.10
Proline	0.28	0.32
Histidine	0.20	0.02
Lysine	0.20	0.03
Arginine	0.47	0.60
Cysteine	0.00	0.01
Methionine	0.11	0.03
Phenylalanine	0.63	0.94
Tyrosine	11.52	9.58
total	99.70	99.71

When analyzed by X-ray diffraction, the FS sample showed a major peak at 20.5 degrees, which corresponded to the specific crystalline spacing of 0.439 nm, a characteristic feature of silk fibers with a highly oriented β structure. The intensity of the peak in the FS-F group was higher than that in the FS group, indicating that the crystallinity of the silk fibers increased upon fibroin treatment. On the contrary, the crystallinity of the silk fibers seemed to decrease upon the EGDE treatment (Figure 3). Our observations are consistent with previous studies (Asakura 1985; Gotoh 1993). EGDE-induced crosslinking might account for the structural changes of proteins in silk fabrics, leading to more random coils and wider amorphous areas.

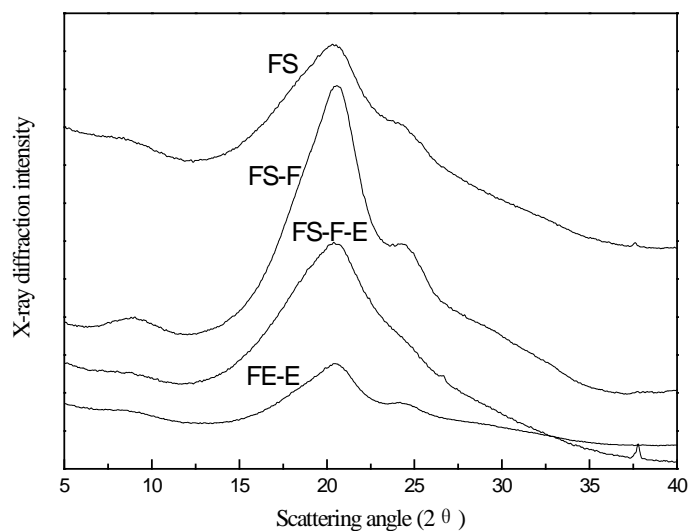


Figure 3. X-ray diffraction intensity curves of FS, FS-F, FS-F-E and FE-E.

TGA was used to analyze the thermo stability of samples with and without fibroin-EGDE treatments. We measured two inflection points, one at 100°C and another at 280°C (Figure 4). The temperature at which the peak of weight loss of the FS-F-E sample was higher than that of the FS sample (333.94°C vs. 326.411°C), indicating the fibroin-EGDE treatment increased the thermal stability of the silk fabrics.

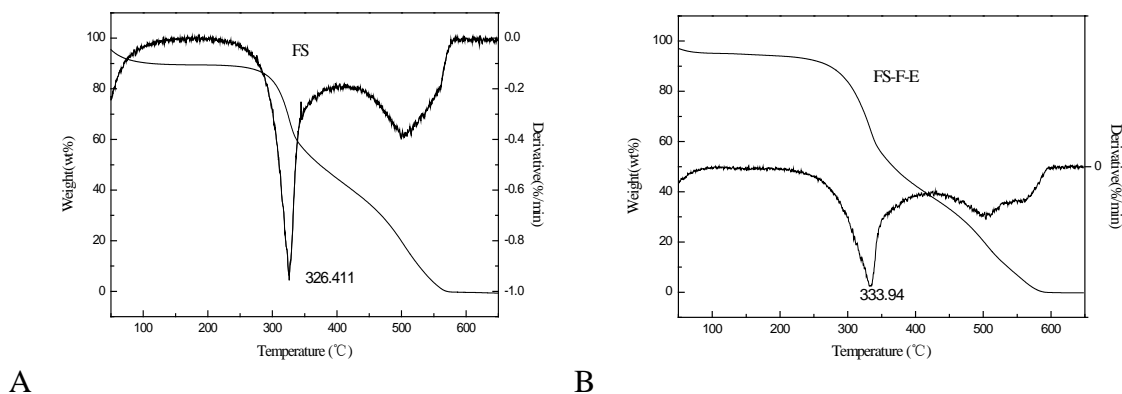


Figure 4. TGA curves of FS (A) and FS-F-E (B).

SEM

Microstructures of silk fabrics were observed under SEM (Figure 5). The surface of the FS-F-E sample was smoother and straighter than that of the FS sample. This might result from EGDE-induced crosslinking between the silk fibroin and silk fiber, while silk fibroin might be adsorbed only on the surfaces of silk fibers without EGDE.

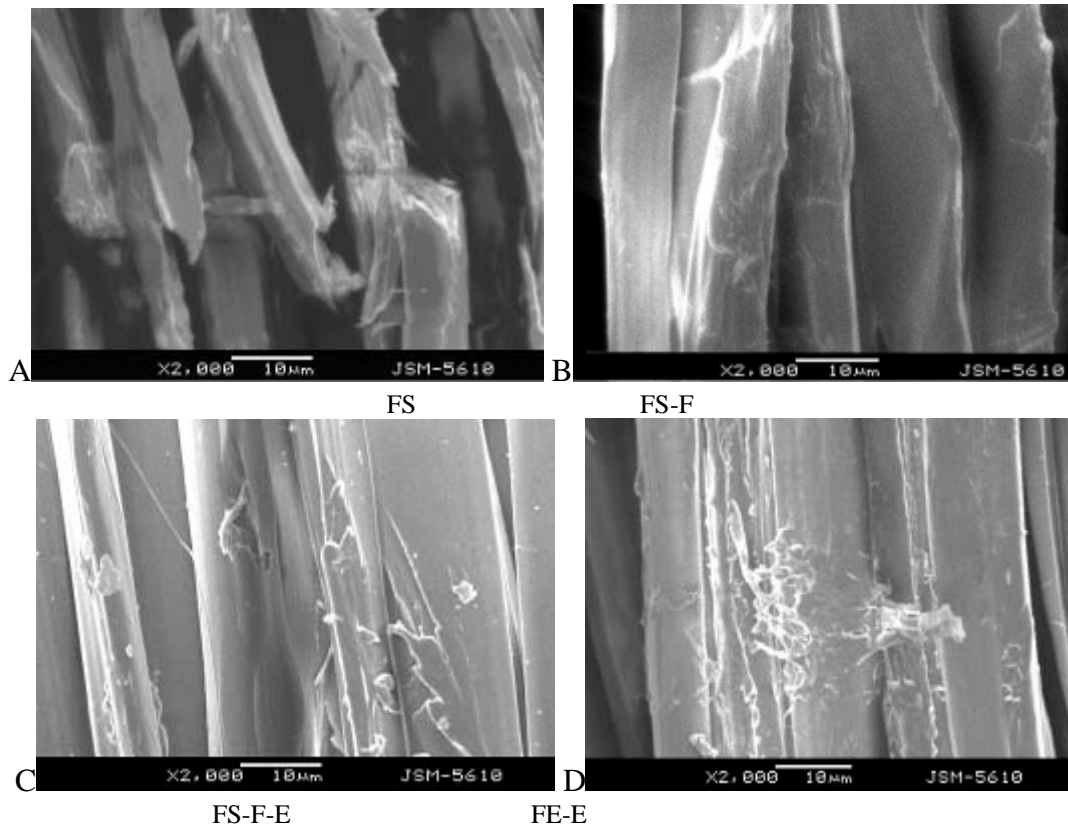


Figure 5. SEM micrographs of FS (A), FS-F (B), FS-F-E (C) and FE-E (D).

Application Case

Encouraged by positive results obtained from the Fibroin-EGDE method that we developed, we tried to use this method to consolidate a silk relic made in the Liao Dynasty, which spanned from 907 to 1125 in northern China (Figure 6). This ancient silk relic was fragile and yellowing, apparently due to deleterious effects of microorganism, dirt, and ageing. The standard process of the fibroin-EDGE treatment was applied onto the silk relic as was done in our previous laboratory experiments.



Figure 6. A silk relic dated to the Liao Dynasty (907-1125).

The physical properties of the silk relic were analyzed before and after the fibroin-EDGE treatment. Due to the fragile nature of the relic, only breaking strength and color change were tested. The consolidation efficacy of the fibroin-EDGE treatment was very high, with strength increasing more than 20 folds (Table 4). The color change was 1.70, which might be due to the uneven color of the original silk relic.

Table 4 Physical property of silk relic

Sample	Strength/ (cN/cm)	Color fastness	Color change
Original silk relic	1.54	/	/
treatment silk relic	35.4	3.41	1.70

The fibroin-EGDE solution's main consolidation material-fibroin has homologous nature with the silk samples, so the ageing products of fibroin will not adversely affect silk relics and not hasten the silk relics' ageing rate. It is very safe to silk relic. When the silk relic is too fragile to withstand traditional needle repair method, fibroin-EGDE solution could be adopted.

Conclusions

In this study, we developed an effective consolidation method, which was based on a combination treatment of fibroin and EGDE, to conserve silk fabric relics. We optimized the consolidation protocol and analyzed the physical and chemical properties of samples with different treatments. It appeared to us that many aspects of the silk fabric relics were improved upon the fibroin-EGDE treatment.

A trial of the fibroin-EGDE treatment on an ancient silk relic was conducted with a dramatic increase of its strength. Moreover, this new method can be conveniently applied at ambient temperature and humidity, with little affect on the flexibility of the silk. Our fibroin-EDGE consolidation method appears to meet the conservation principle "treatment does not change the appearance of the relic", the fibroin-EGDE solution providing a new way for the preservation of cultural relics.

Acknowledgements

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Materials and Suppliers

White silk habutae fabrics: Sichuan Nanchong Liuhe (Group) Corp, Nanchong, China;

Bombyx mori silk fiber: Zhejiang Misai Silk Co. Ltd , Jiaxing, China;

EGDE: Nagase ChemteX Corporation ,Tokyo, Japan;

YG065 strength tester : LaiZhou Electron Instrument Co., Ltd., China;

SC-80C automatic colorimeter: Beijing kangguang Instrument Co., Ltd., China;

LLY-01 Electronic Instrument Stiffness: LaiZhou Electron Instrument Co., Ltd., China;

Infrared absorption spectra : Nicolet, America;

¹³C NMR spectra : Bruker, Germany;

X-ray diffractometry: Thermo ARL, Switzerland;

Amino acid analysis: Hitachi, Japan;

Scanning electron microscopy: JEOL, Japan;

Thermogravimetric analysis: Pyris, America;

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