Journal of Cultural Heritage 15 (2014) 18–25

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Original article

Determination of the experimental conditions of the transglutaminase-mediated restoration of thermal aged silk by orthogonal experiment

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A R T I C L E I N F O

Article history:
Received 16 June 2012
Accepted 4 December 2012
Available online 16 January 2013

Keywords:
Transglutaminase
Historic silk
Restoration
Biopolymer
Experimental conditions

A B S T R A C T

Unlike the traditional methods of silk restoration based on the application of synthetic polymers, transglutaminase-mediated polymerization was used as a compatible and innocuous method to reinforce the aged silk fabrics. Artificially aged (dry thermal ageing) silk samples were restored using this method. The optimal experimental conditions of the treatment were determined by orthogonal experiment. The effects of the reaction on silk fibers were investigated by tensile tests, TGA and SDS-PAGE. The results showed that tensile strength, elongation rate at breakage and thermal stability of the silk samples were remarkably improved by using this method. Biopolymers with a molecular weight of more than 260 kDa were formed in the reaction. From the results of this study, the potential of transglutaminase-mediated polymerization to restore historic silk was demonstrated.

1. Research aims

The aim of this research was to propose transglutaminase-mediated restoration as a novel method applied to aged silk. The major advantages of this technique were innocuity and compatibility with silk, instead of the traditional silk restoration methods based on the application of synthetic polymers (usually vinyl acetate-derived polymer, acrylic polymers and miscellaneous synthetic thermoplastics) [1]. The restoration effects differ remarkably according to the variation of the experimental conditions. Thus, an orthogonal experiment was carried out to determine the optimal experimental conditions of the treatment. Tensile tests, TGA and SDS-PAGE were performed to investigate the effects of the reaction on silk fibers. This work suggests that transglutaminase-mediated polymerization is a potential method for the restoration of vulnerable historic silk.

2. Introduction

Silk is one of the most significant inventions in the history of human civilization. Numerous archaeological excavations have proved that sericulture emerged in the Yellow River Basin and the Yangtze River Basin as early as 5000 years B.P. In approximately the third century B.C., silk began to be transported to other countries through the Silk Road. This promoted cultural and economic communications, and made outstanding contributions to the development of human civilization [2]. Due to the long history of silk culture in China, a large number of valuable and fabulous silk fabrics were discovered in various ancient tombs.

Silk is mainly composed of fibroin and sericin. In sericulture, the majority of silk is subjected to a degumming process in which the sericin is removed. This involves treatment with aqueous alkali (calcium oxide or plant ash) or lytic enzymes (e.g. from hog pancreas or white gourd) [3]. Thus, most of the silk fabrics were made of degummed Bombyx mori silk, which is mainly composed of fibroin. A long time after burial, the condition of most historic silk fabrics is poor; some even turn into powder when touched. Thus, there is an urgent need to develop appropriate conservation techniques to restore the mechanical strength and ensure the long-term preservation of these fabrics.

A wide variety of methods, including weave, mount, silkscreen, polymer, and graft copolymerization [4–9] are commonly and efficiently adopted to consolidate historic textile. Among these, synthetic polymers have been widely used as consolidating agents [10–17] and adhesives [18–21] because of their good mechanical and bonding properties. However, there are some potential problems in the application of synthetic polymers in the conservation and restoration of historic silk. They are not only chemically incompatible with silk protein, but also often applied with toxic materials. Therefore, attempts have been made to replace synthetic polymers with protein solutions and cross-linking agents [22–24]. However, the improper cross-linking agents will bring potential harm to historic silk as time elapses, considering their corrosion and toxicity. Thus, a more promising method of restoring fragile historic silk is based on transglutaminase-mediated protein polymerization.

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1296-2074/$ – see front matter
http://dx.doi.org/10.1016/j.culher.2012.12.002

Table 1
Mechanical properties of artificially aged samples, genuine historic sample (3000 B.P.) and non-aged samples.

Mechanical properties	Artificially aged samples	Genuine historic sample	Non-aged samples
Tensile strength (N)	0.38	0.41	282.75
Elongation rate at breakage (%)	1.53	1.57	12.65

Transglutaminases (EC 2.3.1.13) are a large family of enzymes [25–27] that catalyze a post-translational modification of proteins by forming covalent cross-links between glutamyl and lysyl residues. This leads to increased protein stability and resistance to chemical and proteolytic degradation [28]. It has been reported that transglutaminase can mediate sodium caseinate polymerization to form biopolymers larger than 198 kDa [29]. These biopolymers have been found to significantly improve the tensile strength of sodium caseinate films [30]. As there is no glutamine residue in silk protein, transglutaminase alone has no effect on silk. The addition of sodium caseinate would allow the formation of biopolymers, which could fill in the cracks of silk fibers without harmful agents.

As far as one knows, transglutaminase-mediated polymerization has not yet been used in the conservation domain. The present study has demonstrated the capability of this reaction to restore historic silk. Due to their rarity and irregular shape, genuine historic silk fabrics were not appropriate for a large number of repeated tests. Instead, artificially aged silk samples were used as substitutes for the restoration experiments. The previous work demonstrated that dry thermal ageing of modern silk samples under certain conditions can produce both proper residual tensile strength and chemical characteristics similar to that of genuine historic silk [31]. Therefore, the artificially aged samples used here were prepared using the same method. To determine the optimal experimental conditions, an orthogonal experiment was performed. The polymerization and effects of restoration were characterized by means of tensile test, thermogravimetric analysis (TGA) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

3. Materials and methods

3.1. Materials

Plain weaved modern natural silk textiles were purchased from Hefei Guanghua Silk Co. Ltd. Transglutaminase was purchased from Beijing Biotopped Science & Technology Co. Ltd, the measured activity of which was 200 U/g. Sodium caseinate was purchased from Sinkiang Kerui Dairy Co. Ltd.

3.2. Dry thermal ageing

The purchased natural silk samples were placed on an enamel tray in a forced convection oven, and exposed to a temperature of 230 °C for 24 hours. Then the silk strips were transferred to a dryer at room temperature. The mechanical properties of the artificially aged samples, genuine historic sample (3000 B.P.) and non-aged samples are shown in Table 1 [31].

3.3. Restoration process

The artificially aged samples were restored in the following way: a certain amount of sodium caseinate and transglutaminase were dissolved in 0.1 M Tris-HCl buffer, pH 7.0. The obtained mixture was sprayed onto the silk samples; the sample area to mixture volume ratio was 1000 mm^2: 1 mL. The treated silk samples were then incubated at a certain temperature for a set time. After treatment, the samples were immersed in distilled deionised water (liquor ratio 1:20) for 10 minutes to remove the residual reagent.

3.4. Orthogonal experiment

In the present study, the experiments were based on an orthogonal array [L_{16} (4^4) matrix] where the following four variables were analyzed: incubation temperature (factor A), sodium caseinate concentration (factor B), incubation time (factor C) and transglutaminase to sodium caseinate ratio (factor D). An L_{16} (4^4) matrix, which is an orthogonal array of four factors and four levels, was employed to assign the considered factors and levels, as listed in Table 2. The tensile strength, elongation rate at breakage and color difference of the restored silk samples served as the evaluation indices. Optimal conditions were obtained after the orthogonal experiment and subsequent data analysis. Finally, the experiment was repeated under the optimal conditions in order to verify the data. The control sample did not include transglutaminase. Then, the polymerization on the silk samples was demonstrated by TGA and SDS-PAGE.

3.5. Range analysis

There are two important parameters in the range analysis: K_{ijk} and R_{ik}. K_{ijk} is defined as the sum of the evaluation indices (k, k = X, Y, Z) of all levels (j, j = 1, 2, 3, 4) in each factor (i, i = A, B, C, D), and k_{ijk} (mean value of K_{ijk}) is used to determine the optimal level and combination of factors. The optimal level for each factor considering evaluation index k could be obtained when k_{ijk} is the largest. R_{ik} is defined as the range between the maximum and minimum value of k_{ijk} and is used for evaluating the importance of the factors to each evaluation index. That is, a larger R_{ik} means a greater importance of the factor to index k [32]. Take the L_{16} (4^4) matrix, for example, the

Table 2
Levels and factors affecting the properties of the restored silk samples.

Level	Factors			
	A Incubation temperature (°C)	B Sodium caseinate concentration (%)	C Incubation time (h)	D Transglutaminase to sodium caseinate ratio (U/g)
1	25	0.5	0.5	5
2	37	1	1	10
3	50	2	2	20
4	60	3	3	30