Transglutaminase-Mediated Crosslinking of Gelatin onto Wool Surfaces to Improve the Fabric Properties

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Received 10 September 2008; accepted 11 February 2009
DOI 10.1002/app.30300
Published online 28 April 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: In this study, transglutaminase (TGase)-mediated crosslinking of gelatin on the surface of wool and its effect on the properties of wool fabric were investigated. For the wool fabric used in this study, gelatin (3 g/L) treatment for 1 h combined with 20 U/g of fabric microbial TGase reduced the area shrinkage of $KMnO_4$-pretreated wool fabric from 6.53 ± 0.06 to 1.92 ± 0.15%, which was more effective than that treated with gelatin alone (in which the area shrinkage was reduced to 4.02 ± 0.10%). At the same time, the tensile strength recovered from 267 ± 2.0 to 335 ± 2.1 N. The antifelting ability of treated wool fabric exhibited better washing durability. Scanning electron micrographs showed that the gelatin material smoothed the wool fiber surface by coating or filling the raised scales of the wool with TGase. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 2598–2604, 2009

Key words: enzymes; fibers; modification

INTRODUCTION

Wool is considered as an extremely complex, highly crosslinked protein fiber. The fiber is surrounded by cuticle scales that overlap in one direction; this has traditionally been considered the main reason for the felting shrinkage of wool. Enhancing the smoothness of the cuticle scales can reduce the fiber friction when the fibers move against the cuticle scale direction and to the fiber tip and provide the fabric with antishrinking or antipilling characteristics.[1] The typical commercial shrink-resistant finishing used for decades has been the chlorine–Hercosett process. This effective process, widely used even today, consists of a strong acid chlorine treatment with a subsequent neutralization and an application of polymer resin.[2] A big disadvantage of the process is the problem of environmental pollution, for example, the disposal of absorbable organic chlorides. Hence, there is an urgent need for the development of environmentally friendly processes for wool shrink-resistant finishing.

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Contract grant sponsor: Youth Foundation of Jiangnan University; contract grant number: 20070357.

Contract grant sponsor: National High Technology Research and Development Program of China; contract grant number: 2008AA02Z203.

Wool mainly consists of proteins and lipids, so proteases and lipases have been investigated for wool-fiber modification.[3,4] Current enzymatic processes, especially those using proteases, are difficult to control and are not sufficiently predictable or reproducible on an industrial scale. During such treatments, proteases can easily penetrate into the fiber cortex and cause severe degradation at the macrofibril level and unacceptable tensile strength loss. The application of proteases alone is, therefore, not yet widely used industrially.[4] In addition, protease treatment processes with chitosan, a useful carbohydrate in wool modification, have been reported.[1,5] Recently, some nonproteolytic enzymes, such as transferases [i.e., transglutaminase (TGase)] and oxidases (i.e., laccase, peroxidase, and tyrosinase) have been able to modify the chemistry of protein fiber without breaking the peptide bond.[4,6,7] Among the transferases, TGase (protein–glutamine–glutamyltransferase, Enzyme Commission (EC) 2.3.2.13) is an enzyme capable of catalyzing acyl transfer reactions by introducing covalent crosslinks between proteins and peptides and various primary amines, as illustrated in Figure 1,[8] which can lead to enhanced protein stability. The TGases in different organisms, including mammals, plants, fish, and microorganisms, are involved in several biological processes.[9,10] At this time, TGase is frequently used to improve the functional properties of food proteins. In recent years, it has been found that TGases can remediate damage caused by the action of chemicals and proteases, prevent strength loss, and maintain color during wool processing.[4,11,12]

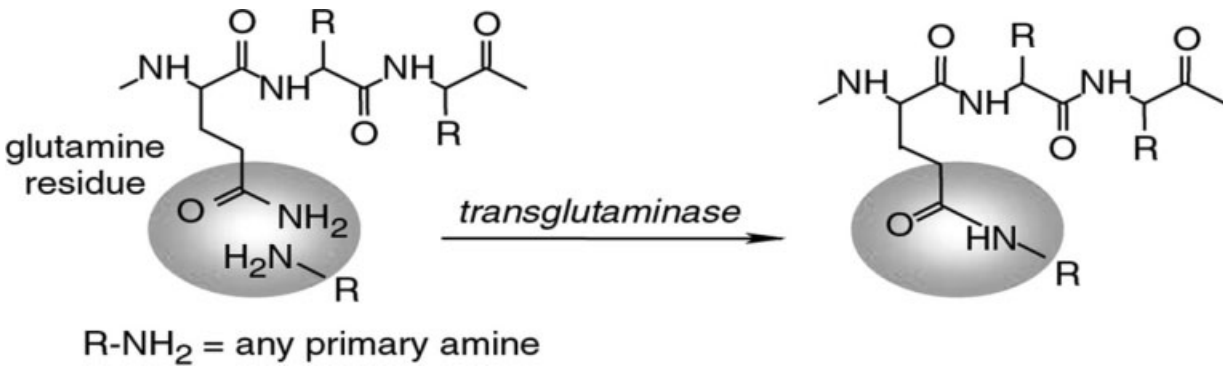

Figure 1 Crosslinking reaction mechanism catalyzed by TGase.

It was of interest to us to investigate whether it was possible to change the functionality of wool with active compounds or proteins for grafting or coating onto the fiber surface in the presence of TGase. Gelatin is a linear polymer and a good substrate for TGase. It is one of the most convenient proteins and is used to manufacture biodegradable and edible films and coatings of food.[13] Gelatin is also usually used as a biomaterial for wound dressing or as a scaffold for tissue engineering.[14] The aim of this study was to examine the effect of gelatin on the dimensional stability and mechanical properties of wool with TGase-mediated crosslinking. The surface morphologies of the wool samples were analyzed by means of scanning electron microscopy (SEM).

EXPERIMENTAL

Materials

Wool fabrics

Worsted wool fabrics (Gaberdine, unbleached 100% pure 2/1 twill, made from 32s wool fiber with 410 ends/10 cm × 250 picks/10 cm) were kindly supplied by Wuxi Xiexin Group (Wuxi, China) and were used in all the experiments. Before use, the fabric was treated with a solution containing 1 g/L nonionic detergent at 40°C for 1 h. Then, the fabric was thoroughly washed with water and air-dried.

Reagents

TGase was separated from a culture broth of *Streptomyces hygroscopicus* according to a method reported previously.[15] The measured activity of the TGase preparation used was 100 U/g. The porcine skin gelatin was chemical grade with an average molecular weight of 10×10^4 Da and a hydrophile–lipophile balance value of approximately 9.8 (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). All of the other chemicals used were analytical-reagent grade and were purchased from Sinopharm Chemical Reagent Co.

Methods

Pretreatment of the wool fabrics with hydrogen peroxide (H_2O_2) and potassium permanganate ($KMnO_4$)

Before treatment with gelatin and TGase, the wool samples were pretreated with 1% on weight of fabric (owf) H_2O_2 at pH 9.0 (0.1 mmol/L Na_2CO_3 and 0.1 mmol/L $NaHCO_3$ buffer) for 1 h at 50°C and 4% owf $KMnO_4$ at pH 4–5 for 30 min at 40°C. After pretreatment, the samples were rinsed and dried at room temperature. The owf value was calculated as follows:

$$\text{owf } (\%) = \frac{\text{Weight of chemical}}{\text{Weight of fabric}} \times 100\%$$

Crosslinking of the wool and gelatin with TGase

Worsted wool fabric was kept with different concentrations of gelatin in the absence and presence of TGase (10, 20, and 30 U/g of fabric) in 20 mmol/L phosphate buffer (pH 7.0) for 1 h at 40°C and a liquor to fabric ratio of 20 : 1. Control samples were treated with buffer only (no gelatin and TGase were added). After treatment, the samples were rinsed with water, dried at room temperature, and tested for tensile strength and felting shrinkage. The crosslinking reaction of gelatin catalyzed by TGase was analyzed by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) as described in the following section. The crosslinking of the wool fiber and gelatin caused by TGase was determined with SEM.

SDS–PAGE

The crosslinking reaction of gelatin catalyzed by TGase was carried out at 40°C in 20 mmol/L phosphate buffer (pH 7.0) containing 1% (w/v) gelatin as the substrate and at an enzyme level of 20 U/g of protein substrate. The reaction mixtures were incubated for 1 h. The gelatin solution with inactivated TGase was used as the control sample. We then

stopped the reaction mixtures by directly mixing them with the Laemmli sample buffer (2×) containing SDS and dithiothreitol and analyzed them with SDS–PAGE according to the method of Laemmli.[16] A 12.5% separating gel was used. The proteins were stained with a 0.1% solution of Coomasiie Brilliant Blue R-250.

Tensile strengths of the fabrics

The tensile strength of fabrics was measured by a YG (B) 026D-250 fabric tensile strength tester (Wenzhou Darong Textile Instrument Co., Ltd., Wenzhou, China) in accordance with method ISO5081. The samples were balanced at 25°C and a relative humidity of 60% for 24 h before testing. The sample had dimensions of 50 × 250 mm^2 after the yarns were drawn out at 5 mm on both sides. The tensile strength of the wool fabrics was only tested in the warp direction, and the results are given as the arithmetic means of three different samples.

Washing tests to assess fabric shrinkage

The dimensions of the testing samples were 200 × 220 mm^2. Before they were treated by any method, the wool fabrics were first relaxed in a Y(B)089A washing machine (Wenzhou Darong Textile Instrument Co.) with the ISO 6330 7A program, as described in IWS test method 31. After different treatments, the wash area shrinkage was determined according to IWS test method 31. Each fabric was washed with the ISO 6330 program 5A six times. The percentage of area shrinkage of the wool fabric was calculated, and the results are the arithmetic means of three different samples.

The durability of the antifelting properties of the fabric is expressed as the percentage of area shrinkage after repeated washing tests, as described previously, for one, two, three, four, and five cycles, respectively.

Fiber surface analysis

The surface morphologies of the wool samples were visualized with an S-3000N/H scanning electron microscope (Hitachi, Japan), operating at a typical accelerating voltage of 10 kV. The samples were sputter-coated with gold for 40 s at 15 mA before the observation.

RESULTS AND DISCUSSION

Analysis of the gelatin catalyzed by TGase with SDS–PAGE

A gelatin-based system was used, and the SDS–PAGE obtained after crosslinking with TGase is shown in Figure 2. On the SDS–PAGE pattern, new protein biopolymers crosslinked by TGase congregated at the interface of the stacking and separating gel or stopped at the upper part of the separating gel because of the increase in molecular weight (lane b). The gelatin proteins were polymerized by covalent crosslinking instead of disulfide bonds because the protein samples were mixed with the Laemmli sample buffer containing SDS and dithiothreitol and heated before electrophoresis, a process that reduces disulfide bonds.

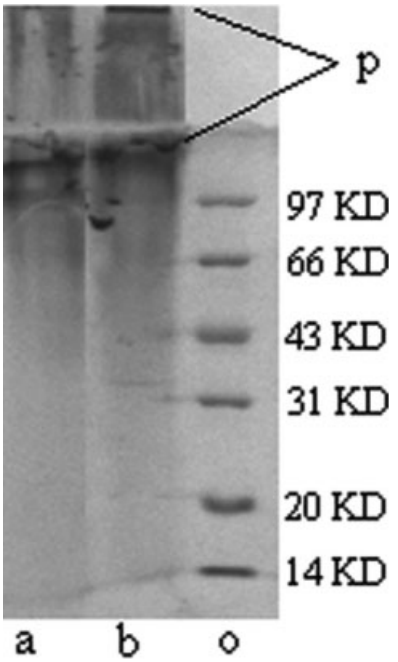

Figure 2 SDS–PAGE analysis of the gelatin catalyzed by TGase: (o) marker, (a) gelatin solution with inactive TGase after incubation for 1 h, (b) gelatin solution with TGase after incubation for 1 h, and (p) biopolymers.

Effect of the gelatin on the area shrinkage and tensile strength of the wool fabric after different oxidant pretreatments

The effect of gelatin on the properties of the wool fabric is shown in Figure 3. The percentage of area shrinkage and tensile strength of the wool fabrics without any pretreatment were not obviously affected by gelatin treatment. The area shrinkage only decreased from 8.36 ± 0.13% for the untreated fabric to 7.21 ± 0.06% after treatment with 0.5 g/L gelatin at 40°C for 1 h, and the tensile strength hardly underwent any change, probably because the wool fiber surface was covered by a covalently bound fatty layer, which was responsible for the strong hydrophobicity of the fiber. This fatty layer diminished the affinity of the hydrophilic gelatin to the wool fiber. Chemical or proteases treatments are capable of removing some of the hydrophobic

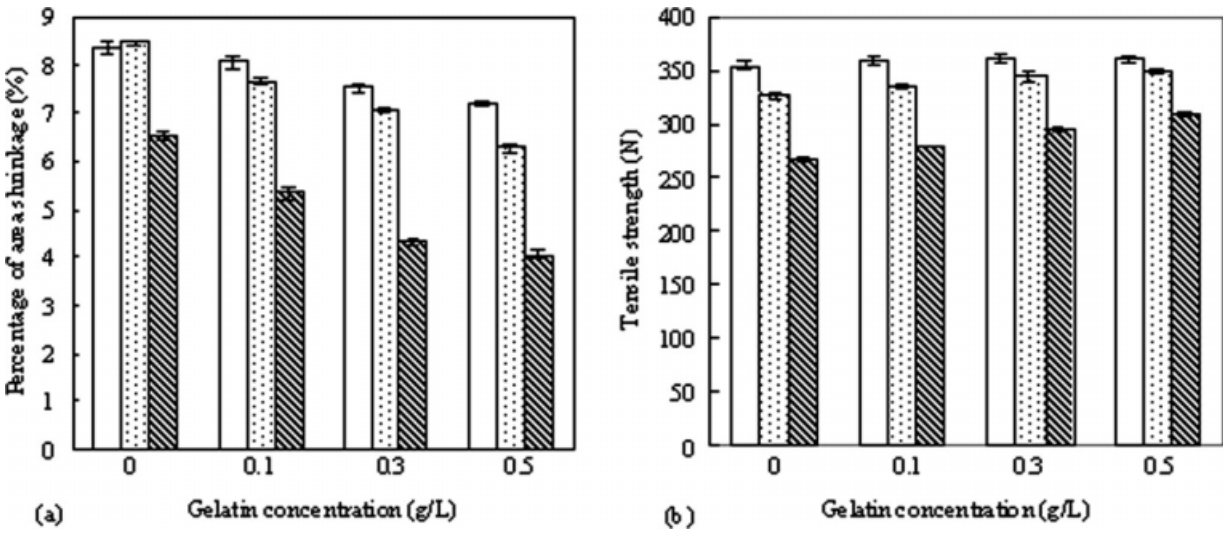

Figure 3 Effects of the gelatin on the (a) area shrinkage and (b) tensile strength of the wool fabrics after different oxidant pretreatments: (□) control, (▨) H_2O_2, and (▨) $KMnO_4$.

substrates to alter the surface properties of the fiber.[17] Accordingly, the hydrophobic nature of the fiber is reduced, and the adhesion of hydrophilic compounds to wool is improved.

$KMnO_4$ is a strong oxidant. It diffuses right through wool fibers and readily oxidizes all of the accessible cystine to increase the wool hydrophilicity. Moreover, the oxidation reaction induced by $KMnO_4$ might cause some damage to the wool surface to improve the antifelting properties of the wool fabric.[18] Figure 3 shows that the $KMnO_4$ pretreatment decreased the percentage of area shrinkage (from 8.36 ± 0.13 to 6.53 ± 0.06%) more efficiently than H_2O_2 pretreatment (scarcely any improvement) but simultaneously resulted in more serious damage to the wool fiber surface and, consequently, the loss of fabric tensile strength (from 355 ± 3.6 to 267 ± 2.0 N). Both $KMnO_4$ pretreatment and H_2O_2 pretreatment enhanced the gelatin–wool affinity, which caused shrink resistance and the recovery of tensile strength for the wool fabric. The percentage of area shrinkage level of the fabric pretreated with $KMnO_4$ further decreased to 4.02 ± 0.05% after the application of 0.5 g/L gelatin, and the tensile strength increased from 267 ± 2.0 to 308 ± 1.9 N. It was suggested that the oxidant pretreatment of the wool fabrics enhanced the efficiency of the following gelatin treatment.

Effect of the gelatin on the area shrinkage and tensile strength of the wool fabric in the presence of TGase after $KMnO_4$ pretreatment

We reported previously that chemical pretreatment could make TGase more accessible to wool keratin to improve the antifelting properties of wool fabric because of protein crosslinking catalyzed by the enzyme.[19] The effect of the gelatin on the area shrinkage and tensile strength of the wool fabric combined with TGase after $KMnO_4$ pretreatment is shown in Figure 4. Both TGase treatment and gelatin treatment alone improved the shrink resistance and remediated the tensile strength loss of the fabric induced by $KMnO_4$ pretreatment. The percentage of area shrinkage of the fabric further decreased slightly, and the tensile strength recovered to some degree after gelatin treatment with increasing TGase concentration. As a result, the percentage of area shrinkage of the fabric after $KMnO_4$ pretreatment decreased to 1.92 ± 0.15%, and the tensile strength increased to 335 ± 2.1 N after treatment with 3 g/L gelatin combined with 20 U/g of fabric TGase at 40°C for 1 h. This implied that chemical pretreatment might have also made the crosslinking of wool keratin and gelatin easier.

Durability of the antifelting properties of the fabric treated with gelatin in the presence of TGase

The durability of the antifelting ability to washing is one of the major interests of textile researchers and users because wool textiles are submitted to frequent washing. Figure 5 depicts the percentage of area shrinkage of the wool fabric after different washing cycles. The antifelting properties of all of the wool fabrics decreased with increased number of washing cycles, but no evident decrease after the second washing cycle was observed for the samples with $KMnO_4$ pretreatment [Fig. 5(b)]. For the fabric without any pretreatment, the area shrinkage increased rapidly after the first washing cycle, whether the fabric was treated with gelatin in the presence or absence of TGase. The fabric area shrinkage after

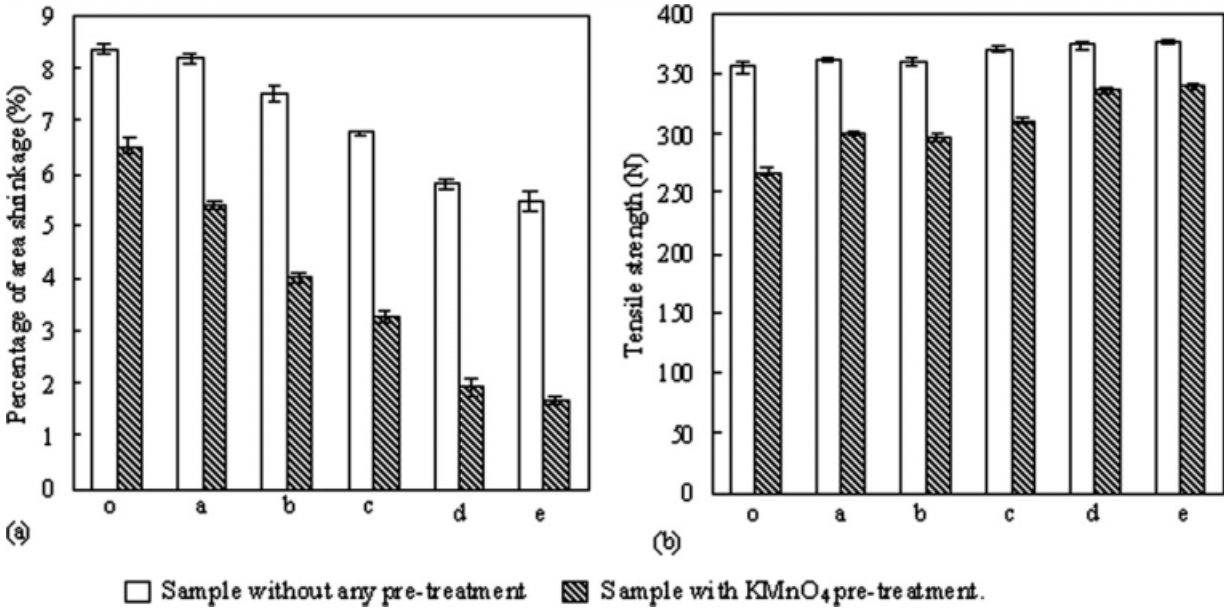

Figure 4 Effects of the gelatin on the (a) area shrinkage and (b) tensile strength of the wool fabrics in the presence of TGase after $KMnO_4$ pretreatment: (o) samples without TGase and gelatin treatment, (a) samples treated with 20 U/g of fabric TGase, (b) samples treated with 3 g/L of gelatin, (c) samples treated with 10 U/g of fabric TGase and 3 g/L of gelatin, (d) samples treated with 20 U/g of fabric TGase and 3 g/L of gelatin, and (e) samples treated with 30 U/g of fabric TGase and 3 g/L of gelatin.

treatment with gelatin in the absence and presence of TGase increased from 5.6 ± 0.2 and 4.7 ± 0.15% to 17.2 ± 0.12 and 16.5 ± 0.1%, respectively; this brought about a twofold to threefold increase after five washing cycles. However, all of the $KMnO_4$-pretreated fabrics obtained better durability of their antifelting properties than that without any pretreatment. The samples treated with gelatin combined with TGase showed better antifelting properties, and the area shrinkage nearly did not change after three washing cycles. A lower area shrinkage of about 2 ± 0.12% was still retained after three washing cycles.

Gelatin, as an excellent film former, can form a three-dimensional network with zones of intermolecular microcrystalline junctions and produce protein films.[20] These forces of protein molecules in gelatin film are much weaker than the covalent bonds. The gelatin film, which could be readily formed on the wool surface, would be easily dissolved into water. So, the results in Figure 5 demonstrate that the wool

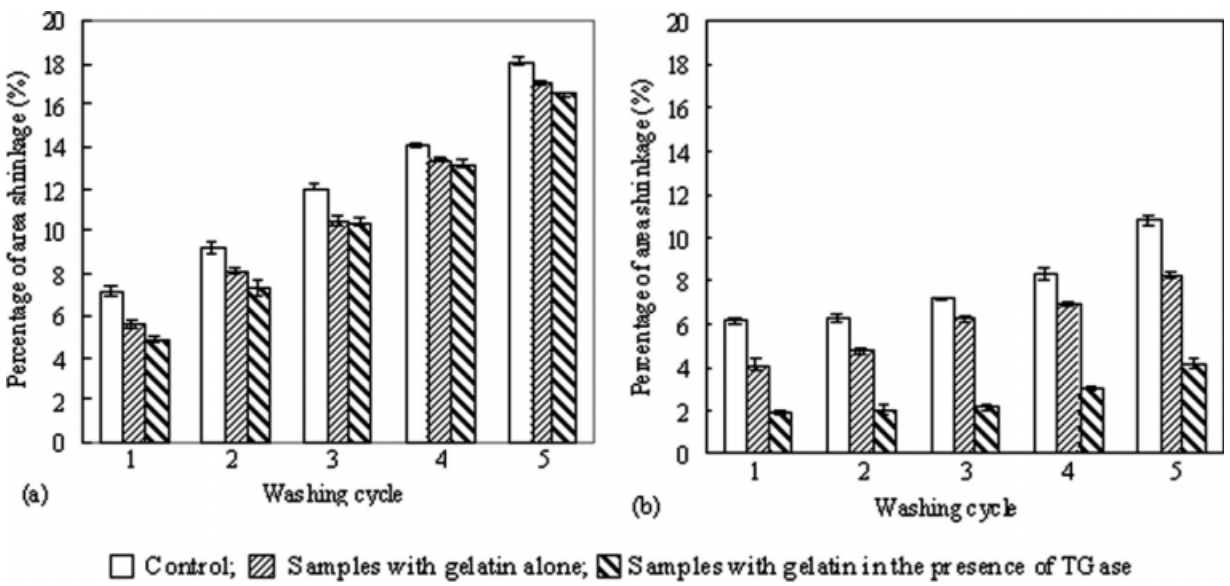

Figure 5 Durability of the antifelting properties of the wool fabrics treated with gelatin: (a) fabric without any pretreatment and (b) fabric with $KMnO_4$ pretreatment.

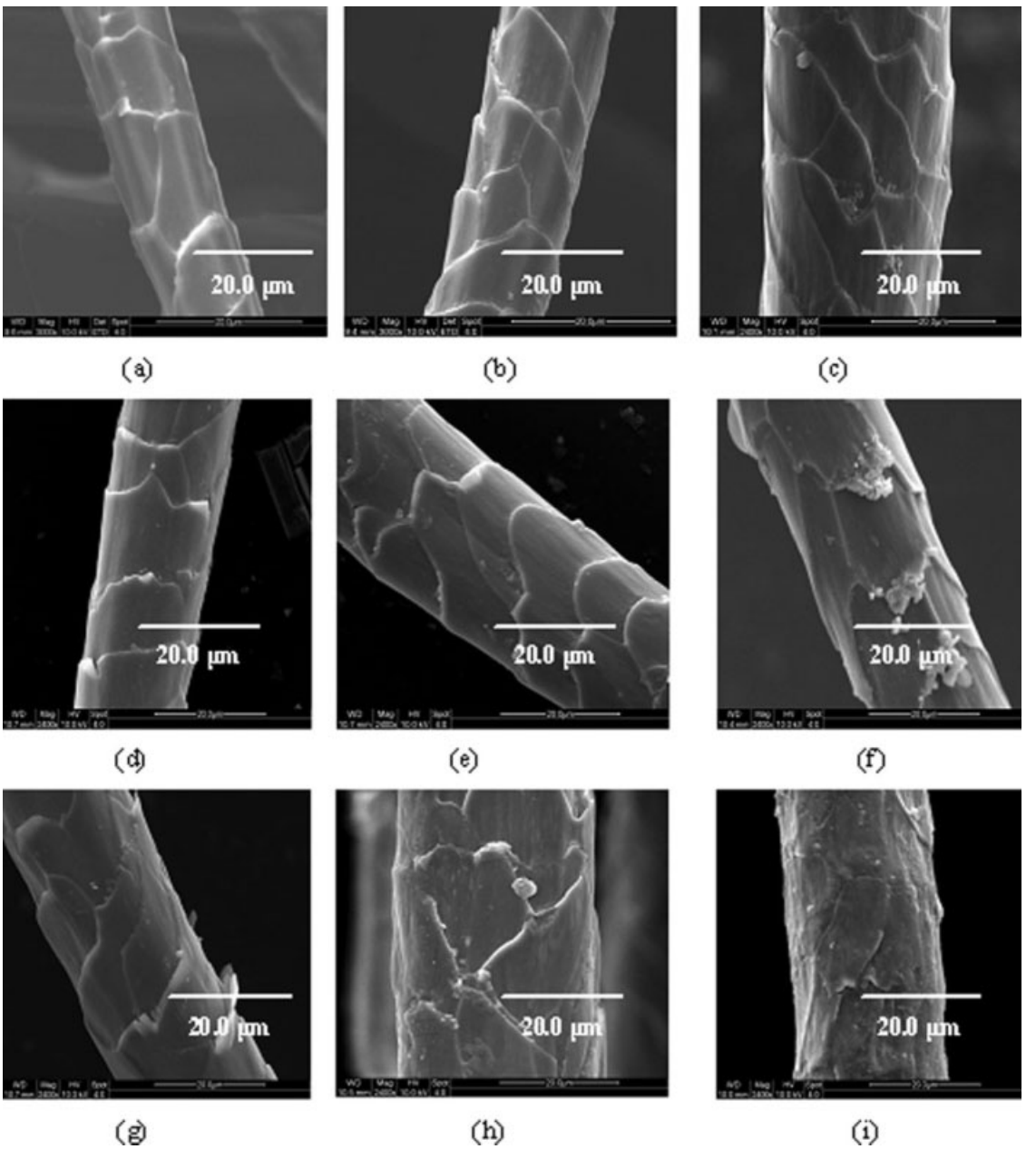

Figure 6 SEM photographs of the wool fiber samples: (a–c) control (without any pretreatment), sample treated with gelatin alone, and sample treated with gelatin combined with TGase; (d–f) sample pretreated with H_2O_2 and H_2O_2-pretrated fabric treated with gelatin alone and with gelatin combined with TGase; and (g–i) sample pretreated with $KMnO_4$, $KMnO_4$-pretrated fabric treated with gelatin alone and with gelatin combined with TGase.

fabric with gelatin treatment alone had a relatively poor washing durability of antifelting. At the same time, gelatin exhibited a strong affinity to $KMnO_4$-pretreated wool in the presence of TGase. Such affinity was possibly because of the covalent crosslinking between the gelatin proteins or between gelatin and wool induced by TGase.

Surface analysis of the treated wool fibers

Scanning electron micrographs (Fig. 6) revealed how the combined system of gelatin and TGase provided antifelting properties to the oxidant-pretreated wool fabrics. As shown in the micrographs in Figure 6, the cuticle scale on the unpretreated wool fiber appeared intact, which is considered the primary cause of felting in wool during an aqueous washing process [Fig. 6(a)]. No morphological changes appeared on the gelatin-treated wool fibers [Fig. 6(b)]. Although the fiber that was treated with gelatin combined with TGase exhibited a surface with very slight alterations, the scales could still be clearly distinguished [Fig. 6(c)]. This was consistent with the results that showed that there was a higher

tendency of felting for the unpretreated wool fibers after gelatin treatment or gelatin combined with TGase treatment. There were no significant changes in the wool surface after H_2O_2 pretreatment, and only some light damage was observed at the edge of the scale by accident [Fig. 6(d)]. Further treatment with gelatin alone did not alter the morphological properties of the wool, but some floccules were found on the surface of the fibers treated with gelatin combined with TGase, which should have been the gelatin polymers crosslinked with TGase [Fig. 6(e,f)]. Pretreatment with $KMnO_4$ resulted in rugosities in the fiber, and the observed damage appeared as a partial lifting of the scale edges, which caused fiber damage. Compared with H_2O_2, $KMnO_4$ might have oxidized more chemical groups than H_2O_2 and induced much more severe damage to the wool at the given concentration. Some particles were found on the gelatin-treated fabric, and scale edge passivation was obtained [Fig. 6(g,h)]. Gelatin combined with TGase completely smoothed the fiber surface by filling or coating the protuberant scales [Fig. 6(i)], which removed the directional friction effect of wool and helped to prevent interlocking of the scales induced by washing. Then, wool fabrics had better shrink resistance.

CONCLUSIONS

Gelatin combined with TGase, which is known to catalyze the crosslinking reaction by forming intermolecular and intramolecular isopeptide bonds between proteins, was used to modify the surface properties of wool fabric. After $KMnO_4$ pretreatment, the area shrinkage of the wool fabric decreased from 6.53 $\pm$ 0.06 to 1.92 $\pm$ 0.15% by treatment with 3 g/L of gelatin in the presence of 20 U/g of fabric TGase. The antifelting abilities of the treated wool fabric presented better washing durability. The gelatin–TGase treatment remediated the fiber damage resulting from $KMnO_4$ pretreatment. This was confirmed by the determination of the tensile strength of the fabric, which recovered from 267 $\pm$ 2.0 N after pretreatment to 335 $\pm$ 2.1 N. SEM provided evidence that the gelatin was successfully coupled to the wool surface with TGase to smooth the wool fiber surface by coating or filling in the scales. These results provide an alternative, environmentally friendly process to functionalize wool surfaces by means of TGase for grafting or coating the fiber with active proteins or compounds.

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