

The Silk Textile Embedded in Silk Fibroin Composite: Preparation and Properties

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Electronic Supplementary Information

Abstract Silk reinforced silk-fibroin-based composites were prepared by embedding of silk textile into regenerated silk fibroin (RSF) matrix. The breaking stress and breaking strain of the composites were found 37.7 MPa and 71.1% respectively at $(95 \pm 5)\%$ RH. Morphological analysis was carried out to observe fracture behavior of the samples. The *in vitro* biodegradation test showed that the composite degraded slowly and lost 70% weight at the end of 168 h. Moreover, compared with RSF pure film, the composite kept strength and toughness much longer time. In conclusion, this composite has the potential for more accurate cytology research and biomedical tests in the future.

Keywords Silk protein; Biomaterials; Composite; Mechanical properties; Natural product

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INTRODUCTION

Bombyx mori silk has remarkable biocompatibility, *in vivo* biodegradability and low immunogenicity, which has been fabricated into many kinds of biomaterials, *i.e.* films, gels, scaffolds^[1–4]. Although regenerated silk fibroin (RSF) film could be obtained by dissolving fibers in salt solution and subsequent processes^[5], the mechanical properties of this two dimensional RSF film are quite different from those of one dimensional natural silk fiber. The fiber shows high toughness while the film is brittle in dry condition ($(50 \pm 5)\%$ RH). Moreover, the breaking stress of film decreases quickly in wet condition ($(95 \pm 5)\%$ RH). It is well known that water plays a role of plasticizer to alter molecular interactions, *i.e.* hydrogen bond^[6, 7], therefore the swollen RSF film changes from rigid to rubber-like material with longer elongation and smaller strength, which is unlikely to be applied in most biomedical application.

Blending is an efficient way to improve mechanical property of materials^[8, 9]. Silk fibers, which have excellent mechanical properties, have been used to reinforce a number of synthetic polymers, such as PLA, PEO, PBST^[10, 11]. However, these matrix polymers may be either cytotoxic or incompatible with the reinforcing fibers. In our previous work, a silk fiber/silk fibroin composite was successfully

prepared *via* a solution casting process^[12]. There was good compatibility between reinforcing fibers and matrix. However in the model the composite was tough along the fiber direction, while it was discussed only in dry condition.

The aim of this work is to prepare an all silk composite which could be used as repair material. To produce a more isotropic composite, we employed a bidirectional knitted silk textile instead of silk fiber to reinforce silk fibroin matrix. The present investigation involves preparation of composites and measuring the mechanical properties especially in wet condition since a reasonable mechanical strength is necessary for repair material. The thermal stabilities and biodegradability of the composites were also measured since long-term existence is an important factor for selecting medical repair materials.

EXPERIMENTAL

Preparation of Silk Textile/RSF (ST/RSF) Composite

Briefly, *Bombyx mori* cocoons were degummed with NaHCO_3 aqueous solution and then dissolved in LiBr aqueous solution. After being filtered, the solution was dialyzed against deionized water for 3 days. The dialyzed solution was concentrated to 12 wt% solution according to the established procedures^[13, 14]. After washed with deionized water, the commercial silk textile was impregnated in RSF solution prior to being completely cast at room temperature and $(50 \pm 5)\%$ RH. The weight ratio of silk textile in the composite was 15%. The produced composite

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was immersed in alcohol for post-treatment and then vacuumed under room temperature and kept in a desiccator before the measurements.

Characterization

The morphology of the fracture sections was observed using a Tescan 5136 MM scanning electron microscope after sputtering with gold. The transmittance of the composite was examined by a Lambda 750 UV-Vis spectrophotometer. The thermal stabilities of composite as well as pure RSF film and silk textile were examined up to 500 °C through Perkin Elmer Pyris-1 thermogravimetric analyzer at a heating rate of 10 °C/min with a purging nitrogen gas stream of 40 mL/min.

Mechanical Testing

Tensile tests were carried out on Instron 5565 universal test machine at room temperature and $(50 \pm 5)\%$ RH or $(95 \pm 5)\%$ RH, respectively. Before test at $(95 \pm 5)\%$ RH, the specimens were immersed in deionized water for 30 min. The dimension of the test specimen was about 40 mm $\times$ 5 mm $\times$ 0.15 mm; the gauge length was 20 mm.

Biodegradability Testing

Biodegradability was determined by treating the composites and RSF film with protease XIV (Sigma-Aldrich) with an activity of 4 U/mg, in 0.1 mol/L phosphate buffer solution (pH: 7.4) at 37 °C^[15] for 6, 12, 24, 72, 120 and 168 h. The concentration of the enzyme solution was set at 0.7 mg/mL. Each specimen was treated in 7 mL solution, which was replenished with fresh solution every 24 h. The specimens were dehydrated in vacuum for more than 3 days.

RESULTS AND DISCUSSION

As shown in Fig. 1(a), with the embedding of silk textile, the produced ST/RSF composite displayed visibly homogeneous and translucent. In the visible region, the transmittance of ST/RSF composite was nearly 90% (Fig. S1, in electronic supplementary information, ESI). The fracture surfaces of composites are shown in Figs. 1(b) and 1(c). In mechanical test, the composites were stretched in two directions, parallel to either fiber array (Fig. 1b1) or at a angle of 45° with both fiber arrays (Fig. 1c), which were defined as Composite-0° and Composite-45° accordingly. The fracture section view of the matrix in composite (Fig. 1b2) was smooth and dense.

Mechanical Properties

The mechanical properties of the prepared composites and RSF film are given in Table 1. It was found that at $(50 \pm 5)\%$ RH, the breaking stress and breaking strain of Composite-0° were a little bit higher than those of RSF film (64.5 MPa and 3% versus 57.9 MPa and 2.5%). At $(95 \pm 5)\%$ RH, the

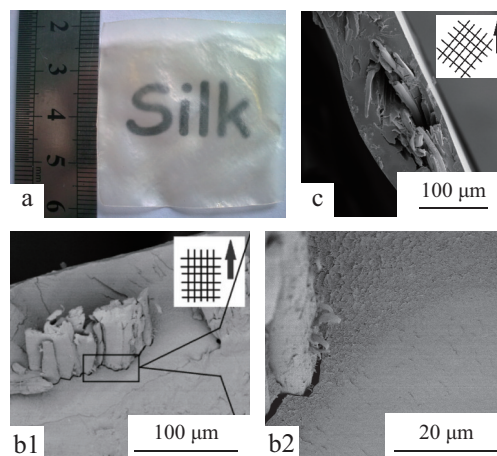


Fig. 1 Photograph of ST/RSF composite (a), SEM images of the Composite-0° (b1, b2) and Composite-45° (c) for the fracture surface after tensile test. In the inserted schemes the arrow points out the stretching direction and crossed grid represents silk textile.

breaking stress of Composite-45° was several times higher than that of RSF film (37.7 MPa compared with 4.3 MPa). In such case, Composite-0° and Composite-45° possessed good performance on mechanical characteristics, especially at the high RH. The reason should lie on the fact that filaments in silk textile have well packed β -sheet region (crystalline region), which could not be affected by water and the good adhesion between matrix and textile also has contribution. Moreover, the breaking strain of Composite-45° was 71.1%, twice higher than that of Composite-0° (31.4%). This was mainly because the resistance to deformation in Composite-45° came from two fiber arrays while in Composite-0° only fibers in the parallel direction bore the tensile force. From this investigation, it is clear that the ST/RSF composites, which were toughened by adding silk textile, gained huge improvement in mechanical properties over pure RSF film especially in wet condition.

Thermal Properties

The thermo-gravimetric analysis (TGA) and differential thermal gravity (DTG) results of composite, RSF film and silk textile are presented in Fig. 2. It was evident that the maximum thermal degradation rate of pure RSF film occurred at around 295 °C, much lower than that of silk textile (325 °C), both of which were consistent with literatures^[16]. The maximum thermal degradation rate temperature of composite was between them, 300 °C. Moreover, both RSF film and composites showed gradual weight loss with the temperature ranging from 40 °C to about 150 °C, while the curve of silk textile was steady. This

Table 1 Mechanical properties of RSF film and composites at different relative humidity ($n \geq 5$)

	Sample	Breaking stress (MPa)	Breaking strain (%)	Breaking energy (*MJ/m ³)
$(50 \pm 5)\%$ RH	RSF film	57.9 ± 3.2	2.5 ± 0.1	0.78 ± 0.07
	Composite-0°	64.5 ± 3.1	3.0 ± 0.2	1.1 ± 0.1
	Composite-45°	45.5 ± 2.9	3.7 ± 0.5	0.95 ± 0.1
$(95 \pm 5)\%$ RH	RSF film	4.3 ± 1.0	180.1 ± 13.8	5.1 ± 0.9
	Composite-0°	27.6 ± 2.4	31.4 ± 2.5	4.5 ± 0.7
	Composite-45°	37.7 ± 1.4	71.1 ± 1.6	13.1 ± 1.7

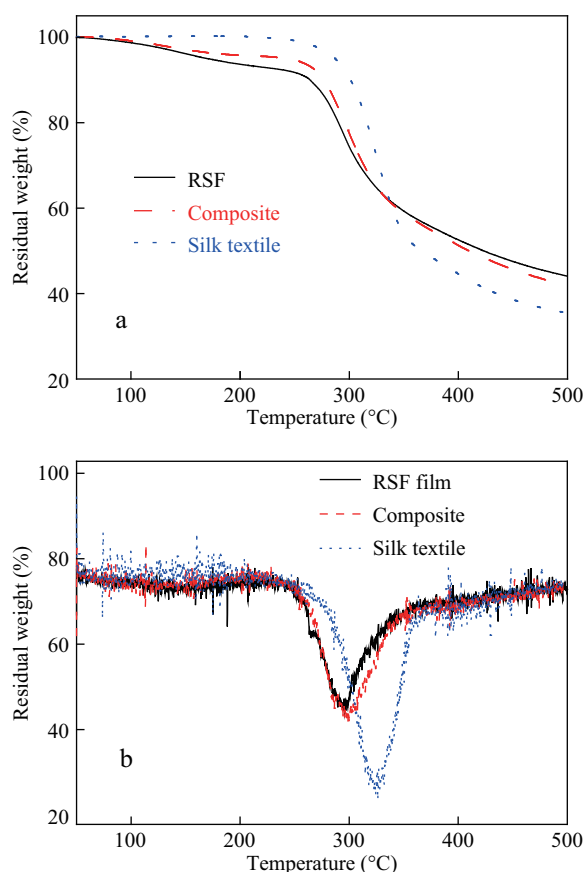


Fig. 2 The TGA (a) and DTG (b) curves of RSF film, silk textile and composite

weight loss due to dehydration prompted that natural silk absorbed less water than RSF materials.

Biodegradabilities

To functionally assess the differences of material stability, *in vitro* degradation tests of RSF film and composites were performed. The weight loss and change of mechanical properties of materials during a degradation period by protease are showed in Figs. S2 and S3 (in ESI). The results indicated that the breaking stress and breaking strain stayed the same during a period of 72 h in PBS solution either for

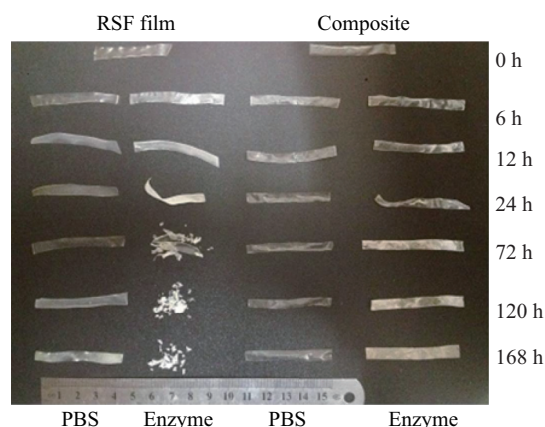


Fig. 3 Change of shapes of materials during a degradation period of 168 h

RSF film or composites (Figs. S3A and S3B, in ESI). Meanwhile, in enzyme solution, there were obvious decreases of the breaking strain for RSF film (from 180% to 80%), while the breaking strain of Composite-0° and Composite-45° did not change a lot. The decrease of breaking stress of Composite-0° (or Composite-45°) occurred at 72 h, dropping to half of the original one. The fracture section view of the composite (Fig. S4, in ESI) showed that matrix in composite became loose. At the same time RSF film has fallen into pieces, as observed by naked eye. The change of shapes of materials during test is displayed in Fig. 3. Therefore it can be presented that after degradation composite still keep some strength and toughness when RSF film vanished to a certain degree.

CONCLUSIONS

In this paper, an all-silk protein material was fabricated by embedding of silk textile into RSF matrix. The results demonstrated that, in wet condition, comprehensive mechanical properties of composite improved over those of pure fibroin films, especially in the tensile direction at a 45° angle with the knitting direction. It is concluded that such an improvement is because of the packed β -sheet region of natural fiber as well as the alignment in textile, and subsequently 45° angle gives the opportunity for the use of vertical and horizontal knitted silk fibers. Those results provide new insights into the preparation of silk protein based materials with promising properties for the potential biomedical applications.

Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at <http://dx.doi.org/10.1007/s10118-018-2117-8>.

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