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Electrospinning and Characterization of Silk Fibroin/Gelatin Nanofibre Mats

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***Abstract*—**In this study, Bombyx mori silk fibroin/gelatin (SF/GT) nanocomposite with different GT ratio (SF/GT 100/0, 90/10 and 70/30) were prepared by electrospinning process and crosslinked with glutaraldehyde (GA) vapor. Properties of crosslinked SF/GT nanocomposites were investigated by scanning electron microscopy (SEM), mechanical test, water uptake capacity (WUC) and porosity. From SEM images, it was found that fiber diameter increased as GT content increased. The results of mechanical test indicated that the SF/GT 70/30 nanocomposites had both the highest Young's modulus of 342 MPa and the highest tensile strength of about 14 MPa. However, porosity and WUC decreased from 62% and 405% for pristine SF to 47% and 232% for SF/GT 70/30, respectively. This behavior can be related to higher degree of crosslinking as GT ratio increased which altered the structure and physical properties of scaffolds. This study showed that incorporation of GT into SF nanofibers can enhance mechanical properties of resultant nanocomposite, but the GA treatment should be optimized to control and fine-tune other properties to warrant their biomedical application.

***Keywords*—**Electrospinning, gelatin, mechanical properties, nanocomposites, silk fibroin.

I. Introduction

TISSUE engineering has been recognized as a new approach for repairing tissue or organ by using bioresorbable synthetic scaffolds that help and promote the development of new tissues in vitro and subsequently implanting them in vivo. In order to assist the formation of new tissue, the fabricated scaffold should follow the extracellular matrix (ECM) structure, which is a fibrous 3D nanoscaled network with an open porosity that provides structural support for developing tissues [1], [2]. In this context, electrospun nanofibers have gained enormous attention due to their distinctive characteristics including large surface area, high porosity with interconnected pores. Besides the fibrous structure can greatly mimic the ECM which has a pivotal role in cell survival, migration and differentiation in addition to the presentation and storage of growth factors and signal detection. In this process, a high electrical voltage is applied to a polymer solution in a finite distance between a capillary and a collecting substrate [3], [4].

Despite the interesting structure of electrospun fibre mats, the material selection for the fabrication of scaffolds is important as well. The natural silk fibre from *Bombyx mori* (*B.mori*) is a protein-based polymer that has been used as high-quality textile fibres and sutures for a long time. It is mainly composed of two class of proteins, fibrous protein so-called fibroin, which forms a thread core, and glue-like proteins known as sericin which coats fibroin. SF can be fabricated to various forms including film, gel, sponge, fibres and so forth [5]. Recently electrospun SF has attracted significant attention in biomedical areas and investigated as scaffolds for various types of tissues such as skin, nerve, bone, cartilage and blood vessels as well as carriers for drug delivery and wound dressing [6]-[8]. This is because of its remarkable properties such as good biocompatibility, controlled biodegradability, good oxygen and water vapor permeability, versatile processability, and minimal inflammatory reaction [9]. Hence, it has attracted significant attention in development of new materials specifically for tissue engineering applications and has been blended with various polymers including poly(vinyl alcohol) (PVA) [10], collagen [11], chitosan [12], GT [13] and so forth.

GT is natural biopolymer obtained by denaturation of collagen from animal tissues such as skin, muscle, and bone. It thus carries many of the favorable properties of collagen like biocompatibility, biodegradability as well as formability and cost efficiency. All these characteristics are responsible for the widespread use of this material in many biomedical applications [14], [15]. Nonetheless, due to poor mechanical properties as well as structural instability upon hydration various approaches such as crosslinking or a structural modification agent or blending with other polymers were applied [16]-[19].

The development of electrospun fibers from SF and GT was reported. Addition of GT into SF not only improved the spinnability of SF but also enhanced mechanical properties because of formation of intermolecular hydrogen bonds and the increment of β-sheet structure in nanocomposite [20]. Chomachayi et al. [21] found that introducing GT to SF enhanced nanofiber's diameter, bulk hydrophilicity, surface wettability, mass loss percentage, however slightly reduction of ultimate tensile strength and Young's modulus were observed. Okhawilai et al. [22] investigated the effect of preparation conditions for electrospun fibre mats of Thai SF/type B GT and reported a decrease in average fibre diameter by increasing applied voltage and decreasing SF content. Additionally, SF degradation is accelerated, and cell proliferation is improved by adding GT to SF scaffolds.

This study aims to develop SF/GT electrospun nanocomposites at different blend ratios and use GA to crosslink the blended structure. The morphology, mechanical properties, porosity and WUC of the blended fibres were

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evaluated.

II. Materials and Methods

Bombyx mori silkworm cocoons were purchased from the Yarn Tree (A quality; The Yarn Tree, Greenville, SC, USA). GT from porcine skin (type A, 300 bloom), GA (grade I, 25% in H_2O), sodium carbonate (Na_2CO_3), lithium bromide (LiBr), glycine, methanol and formic acid were purchased from Sigma Aldrich (NSW, Australia). All chemicals were analytical grade and used as received.

A. SF Preparation

SF was extracted by boiling in 0.02 M Na_2CO_3 for 30 min, followed by rinsing three times and further left to dry overnight. Then SF was dissolved in 9.3M LiBr to prepare the 10 wt.%/v solution that was subsequently dialysed against ultrapure water for 48 h via 12400 MWCO dialysis tubes. Dry SF sponge was collected by freezing extracted silk solutions at –80 °C prior to the lyophilisation.

B. Electrospinning

Spinning solutions were prepared by dissolving SF in formic acid followed by adding different weight ratio of GT into the mixture and stirring for 2 hour to obtain a homogeneous solution. The SF/GT solution with different weight ratio (100/0, 90/10, 70/30 (w/w)) and final concentration of 13 % (w/v) were prepared and loaded into a 10 ml syringe with an 18 gauge blunt tip needle. The electrospinning was operated at the high voltage setting of 16 kV and flow rate of 0.3 ml/h with a needle-to-collector distance of 13 cm.

To enhance the structural stability of SF/GT nanofibrous scaffold, crosslinking process was carried out by exposing the fibres to the vapour of 20% (v/v) GA at room temperature for 6h. After crosslinking, samples were immersed in 0.1 M glycine aqueous solution for 30 min to block the residue aldehyde groups.

C. Characterization

SEM: Fiber morphology was characterized by using a Zeiss EVO 40XVP SEM at an accelerating voltage of 15 kV. The average fiber's diameter of each sample was determined by Image J software through measuring the diameters of 50 randomly selected fibers in each SEM image.

Tensile test: Mechanical properties of nanocomposites were measured on stripes of 10 × 30 mm^2 mounted on paper frames by means of double-sticky tap by a Lloyds EZ50 universal testing machine at crosshead speed of 10 mm/min with ambient temperature of 25 °C and humidity of 65%.

Porosity: The porosity of the SF and SF/GT blended scaffolds was measured as follows (1):

$$\text{Porosity} = \left(1 - \left[\frac{\text{Apparent density of the mat}}{\text{Bulk density of polymer}}\right]\right) \times 100\% \quad (1)$$

where apparent density was estimated by measuring the weight per unit volume (2) and the bulk density of SF and GT were 1.25, 1/35 g/cm^3, respectively. Bulk density of SF/GT blend nanofibers was estimated according to average density from (3).

$$\text{Apparent density } (g/cm^3) = \frac{\text{mass of scaffold(g)}}{\text{thickness of scaffold (cm)} \times \text{area } (cm^2)} \quad (2)$$

$$\frac{1}{\rho_{total}} = \frac{wt\,\%gleatin}{\rho_{gelatin}} + \frac{wt\,\%SF}{\rho_{SF}} \quad (3)$$

WUC: In order to determine WUC of nanofibrous mats, scaffold with 1 × 1 cm^2 were weighted (w_0) and immersed in deionized water at 27 °C for 24 h. The excess water on the surface was removed with filter paper followed by weighting the wet samples (w). The WUC was calculated by (4):

$$water\ uptake\ (\%) = \left(\frac{W - W_0}{W_0}\right) \times 100 \quad (4)$$

III. Results and Discussion

A. Fiber Morphology

Electrospun SF/GT nanofibers with different GT blending ratio were fabricated by electrospinning under optimized condition and stabilized with GA. Fig. 1 shows the morphology of crosslinked SF/GT fibres and it could be seen that all SF/GT mats demonstrated a homogeneous bead-free fiber with a smooth surface. The average diameter and size distribution of electrospun nanofibers for SF/GT 100/0, 90/10 and 70/30 were 403.5±104.67, 422.7±97.11, and 426.4 ±54.04 nm, respectively. It is evident that by increasing GT weight ratio into SF matrix not only the fiber diameter increased but also more uniform fiber diameter distribution obtained. The former can be attributed to the viscosity enhancement of the solution and the latter indicated the improvement in spinability of SF/GT solution with increasing GT ratio [20].

B. Porosity and WUC

Fig. 2 shows that the porosity of GA treated scaffold with value of 62%, 59% and 47% for SF, SF/GT (90/10), SF/GT (70/30) respectively. Protein crosslinked by GA through the reaction of GA aldehyde groups with free amino groups of lysine or hydroxylysine amino acid residues of the polypeptide chains [23]. Since GT has higher number of free amine group relative to SF, hence it can be expected that by increasing GT content the crosslinking density of SF/GT construct enhanced. Consequently, as GT weight ratio increased, more fibres adhesion and twinging were observed, which is also evidenced by SEM morphology (Fig. 1) and thereby reducing the porosity of nanofibers.

WUC of the electrospun fibrous scaffolds were shown in Fig. 2. It is evident that SF had the highest WUC of 405% and WUC was significantly decreased depending on GT ratio. For instance, for SF/GT of 90/10 and 70/30 the WUC value decreased to 350 and 232%, respectively. This is because higher GT ratio provides more amino group for crosslinking which increased the cross-linking degree and enhances the compact degree among molecules, which makes it difficult for water molecules to enter [24]. Moreover, as discussed earlier, decrease in porosity of nanofibrous mats by adding GT in the

SF matrix could also be responsible for lower WUC of SF/GT mats.

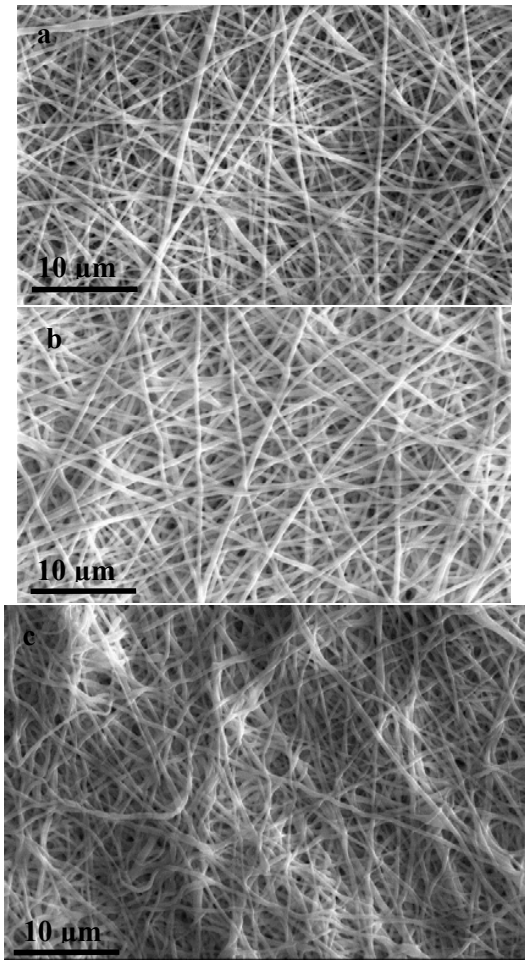

Fig. 1 morphology of the GA crosslinked SF/GT nanofibers (a) 100/0, (b) 90/10 and (c) 70/30

C. Mechanical Properties

Results of tensile test for SF/GT crosslinked mats with different GT ratios were summarized in Table I. It was found that by increasing the GT content, Young's modulus was enhanced from 268 MPa for pure SF to 300 and 342 MPa for SF/GT of 90/10 and 70/30, respectively. Such remarkable improvement could be accounted for higher crosslinking density with incorporation of GT which yielded stiffer and more robust constructs [25]. Moreover, by increasing GT content, porosity was shown a decreasing trend that may lead to more fibre entanglement and contributed to better mechanical properties [26]. On the contrary, it was noted that elongation at break of SF/GT fibers were diminished by increasing GT weight ratio and it reached its lowest value of 16% for SF/GT 70/30 as opposed to 20% for pristine SF. This result indicated that with addition of GT, scaffold became more brittle which is in agreement with the previous finding which reported that adding GT to a polymer matrix reduced the extensibility [27], [28].

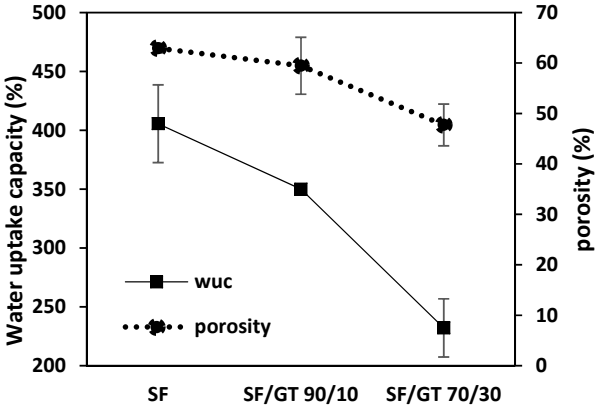

Fig. 2 Porosity and WUC of GTA cross-linked SF/GT constructs at different GT blend ratio

TABLE I
MECHANICAL PROPERTIES OF GA CROSSLINKED SF/GT MATS

Sample (SF/GT)	Young's Modulus (MPa)	Elongation at break (%)
100/0	286	20.47
90/10	300	19.58
70/30	342	16.16

IV. CONCLUSION

SF/GT blended nanofibres with different GT content were successfully prepared by electrospinning and stabilized with GA. Depending on ratio of GT, prepared scaffolds displayed different morphology, porosity, WUC and tensile properties. The mechanical testing data indicated that the SF/GT 70/30 nanocomposites had both the highest Young's modulus of 342 MPa and the highest tensile strength of about 14 MPa. Porosity and WUC decreased from 62% and 405% for pristine SF to 47% and 232% for SF/GT 70/30, respectively. These results could be associated with enhancement in crosslinking degree which caused formation of fused fibres and also rendered the structural integrity of fibre mats. Our study demonstrated that the use of GA allows to modulate the physico-chemical properties of SF/GT fibres, however further study to optimize GA crosslinking is required to obtain stable materials with a wide range of possible biomedical applications.

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