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FT-IR Study of Silk Fibroin/Polyaspartic Acid Scaffolds Prepared by Electrospinning

CHEN QIAO-YAN[1], CHEN GUANG-MEI[2], ZHANG FENG-JUN[2], LI RONG[1] and HUANG YI-PING[1,*]

[1]School of Chemistry and Chemical engineering, Anhui University; Key Laboratory of Environment-Friendly Polymer Materials of Anhui Province, Hefei 230039, P.R. China
[2]School of Material and Chemical Engineering, Anhui Institute of Architecture and Industry, Hefei 230601, P.R. China

*Corresponding author: E-mail: yphuang2001@sina.com; fjzhang@aiai.edu.cn; zhang-fengjun@hotmail.com

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Silk fibroin (SF)/polyaspartic acid (PASP) porous scaffolds were prepared by electro-spinning method with the aim to develop a new scaffold for tissue engineering. In the FT-IR spectra, with the addition of polyaspartic acid, the bands of silk fibroin had no obvious change. By the effects of electro-spinning, the bands of amide II and amide III of silk fibroin shifted to the lower wavenumber which meant the characteristic peaks of the silk II form, obviously, but the strong bands of amide I had no notable change. With the treatment of ethanol the structure of silk fibroin changed more completely and the bands of amide I shifted to the low wave number. The results indicated that there had no effect on silk fibroin's structure when added polyaspartic acid in SF/PASP scaffolds. With the stretch of electro-spinning, the structure of SF/PASP scaffold changed owing to the polymer chains orientation. The silk I form changed to silk II form mostly by the ethanol treatment. The effects of the polyaspartic acid on the morphology of the porous scaffolds were investigated by SEM. The results provided that the addition of the polyaspartic acid had a good effect on electrospinning to make porous scaffolds.

Key Words: Silk fibroin, Electrospinning, Polyaspartic acid, Scaffolds.

INTRODUCTION

Recently, interests on silk fibroin (SF) have been growing as promising biomaterials for its particular characteristics, such as biocompatibility, biodegradability and bioabsorbable. It can be used in wound dressings, vascular grafts and tissue engineering scaffolds[1,2] by blending with other polymers to prepare composites. Researches have shown that hydroxyapatite (HAp)/SF-PLLA films can be made by adding poly-L-lactide (PLLA) to SF solution[3]. The HAp/SF-PLLA composite membrane was therefore a promising GBR membrane with sufficient bioabsorbability. The novel HA/CTS (chitosan)-SF[4] composite was prepared by a coprecipitation method for bone replacement. HA/PA6 3-D scaffolds had been prepared by a method of phase separation and particle leaching combined (PS/PL)[5]. Some other papers have reported[6] that porous scaffolds of SF/collagen (COL) were prepared by the method of electro-spinning aim to develop a new scaffold for vascular tissue engineering.

Porous scaffolds could be prepared by electro-spinning[7], particle leaching[8], freeze-drying method[9] and rapid prototyping technology[10]. Electro-spinning is a simple and effective process of producing polymer fibers in the diameter ranging from several nanometers to several micrometers[11], which has attracted great interest among academic and industrial scientists[12]. Electro-spinning has made it ideal for tissue engineering and human bone marrow stem cell attachment and proliferation[13]. The electro-spinning is also providing researchers with the ability to create, both flat sheets and the seamless tubes required for porous materials. Doshi and Reneker[14] reported the principle of electro-spinning method. The elongation flow and shearing during the spinning facilitates the orientation of the silk fibroin molecule chains and the transform of the soluble silk solution in the gland to the β-sheet found in the spun silk fiber[15]. However, the SF/PASP porous scaffolds were prepared by the method of electro-spinning hasn't been reported, before.

In this work, we prepared SF and polyaspartic acid (PASP) solution by blending the two components directly. SF/PASP scaffolds were prepared by using electro-spinning method. Then, the SF/PASP scaffolds were treated by the ethanol to make sure the structure of SF changed completely. On the other hand, it is helpful to introduce hydroxyapatite in the further study. The conformational features of the electro-spinning method on SF and SF scaffolds were measured by Fourier transform infrared spectroscopy and the morphology of the porous scaffolds was examined by scanning electron microcopy.

EXPERIMENTAL

B. mori silkworm cocoons were provided by Anhui Agricultural Research Institute. Na_2CO_3, LiBr used here were analytical grade and they were purchased commodity. Polyaspartic acid was purchased from Shandong Taihe water treatment Co. Ltd., China.

Preparation of SF/PASP blending solutions: *B. mori* silkworm cocoons were degummed 3 times with 0.5 (w/v) % Na_2CO_3 solutions at 100 ºC for 0.5 h and washed with distilled water in order to remove sericin from the surface of silk fibers. Then testing the sericin whether they were cleaned up or not by using the 2,4,6-trinitrophenol-carmine solution[16]. The washed fibers were dried at room temperature. Dried fibers were then dissolved in a LiBr (9.5 mol/L) solution at 40-60 ºC for 2 h, followed by a dialysis (MWCO 8000-14000) against distilled water for 5 days to remove the salts and small molecules. Centrifugation method was used to get regenerated SF solution. The final concentration of pellucid regenerated SF solution was 2.76 (w/v) %. In consideration of electro-spinning, the regenerated SF solution was concentrated to 30 (w/v) %[17].

Four samples were prepared by dropping different volumes of PASP solution into concentrated SF solution, labeled as PASP00 (SF:ASP = 100:0 (w/w)), PASP05 (SF: PASP = 100:5), PASP10 (SF: PASP = 100:10), PASP15 (SF: PASP = 100:15).

Electro-spinning: The electro-spinning films were prepared by receiving blending fibers on the object slides. Then, the films were dried at room temperature. The dry films were treated by 70 % ethanol for 40 min.

Electro-spinning was performed with steel capillary tube (diameter = 7 mm). The electro-spinning voltage was 40 kV. The perpendicular distance between the grounded metal slice and the syringe tip was set at 20 cm.

FT-IR and SEM analysis: Fourier transform infrared spectra were recorded using a spectrometer (FT-IR: Nicolet NEXUS-870, American). Infrared spectra were taken in the range of 4000-400 cm^{-1} with 2 cm^{-1} resolution.

The SF/PASP scaffolds are platinum coated and examined morphologically by scanning electron microscopy (SEM, S-4800, Hitachi, Japan) with an accelerating voltage of 5 kV.

RESULTS AND DISCUSSION

FT-IR spectra of SF/PASP blending solution before electro-spinning in different proportions are shown in Fig. 1. As shown in Fig. 1, PASP00 has three strong bands at 1652, 1542 and 1241 cm^{-1}. When PASP is added in the SF solution, the peaks of PASP05 are observed at 1654, 1544 and 1243 cm^{-1} and the broad bands at *ca.* 3303 cm^{-1}. These amide peaks are also observed in PASP10 and PASP15 without notable band shifts. The FT-IR spectra of SF's have been reported in some papers[18] and the characteristic bands of silk I (random coil and α-helix) form are *ca.* 3301, 1653, 1543 and 1243 cm^{-1}. As reported in these references, the amide I band at 1652 cm^{-1}, from C=O stretch principal. The band at 1542 and 1241 cm^{-1} is the structure of amide II and amide III, which is from N-H in-plane bending and C-N stretching. And the characteristic broad band of N-H is shown at *ca.* 3301 cm^{-1}. Compared with the pure SF, PASP have no obvious effect on the conformation of SF.

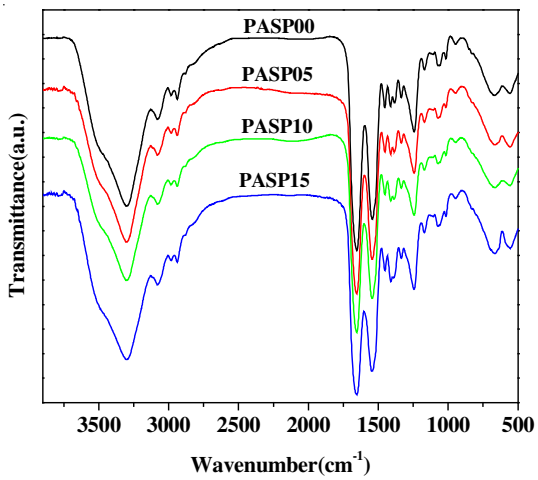

Fig. 1. FT-IR spectra of SF/PASP in different proportions before electro-spinning

After electro-spinning, as shown in Fig. 2, pure SF shows one strong broad bands at *ca.* 3284 cm^{-1} and three strong bands at 1650, 1533 and 1240 cm^{-1}. With the adding of PASP, for PASP05, the broad band shifts to *ca.* 3263 cm^{-1} and widen than the broad band of pure SF. On the other hand, PASP05 has three strong bands at 1646, 1533 and 1238 cm^{-1}. The two peaks slightly shifted to 1646 and 1238 cm^{-1}, which are close to the characteristic peaks of the β-sheet structure. The band at 1533 cm^{-1} (amide II) corresponds to the characteristic band of silk II (β-sheet) form. In Fig. 2, PASP10 and PASP15, with the increase of PASP content, these peaks have no notable band shifts combined with PASP05. The results suggested the conformation of SF in samples transferred from random coil and α-helix to β-sheet, partly. During the electro-spinning process, adding the PASP has an effect on the change of the SF conformation on some degrees. It can be explained by the process of the electro-spinning method. In the process of electro-spinning, the SF solution is solidification at a slow rate from water with the short travel time of the jet in air; the high elongation rate during the electro-spinning process facilitates the SF macromolecules are stretched and rearrangement and the orientation of the silk fibroin chain[19] (Fig. 3). This has an effect on the conformation of SF/PASP blends transition. For PASP00, the broad band at *ca.* 3284 cm^{-1} in Fig. 2 is narrow than the broad band at *ca.* 3301 cm^{-1} in Fig. 1 and the shoulder band at *ca.* 3500 cm^{-1} gets weakened. This phenomenon is also found when PASP add in. It indicates that electro-spinning method may plays a role in the enhancement for hydrogen-bonded interaction. In the skeletal stretching region (1100-900 cm^{-1}) special, the two peaks at 1072 and 1016 cm^{-1} arise from the-(Gly-Gly)-and-(Gly-Gla)-periodic sequences[20].

In order to introduce hydroxyapatite in the further study, the scaffolds are treated with ethanol to change conformation. After electro-spinning, the dry electro-spinning films were treated by 70 % ethanol for 40 min. As shown in Fig. 4, the peaks of PASP00 at 1627, 1527 and 1238 cm^{-1} appeared, indicating that exposure to ethanol induces the β-sheet formation in silk fibroin. Beyond that, the band at 3284 cm^{-1} is very sharp and the shoulder peak at *ca.* 3500 cm^{-1} gets weakened. In the magnifying FT-IR spectra, there is a new band appeared at 1699 cm^{-1}. With the treatment of ethanol, all the scaffolds with

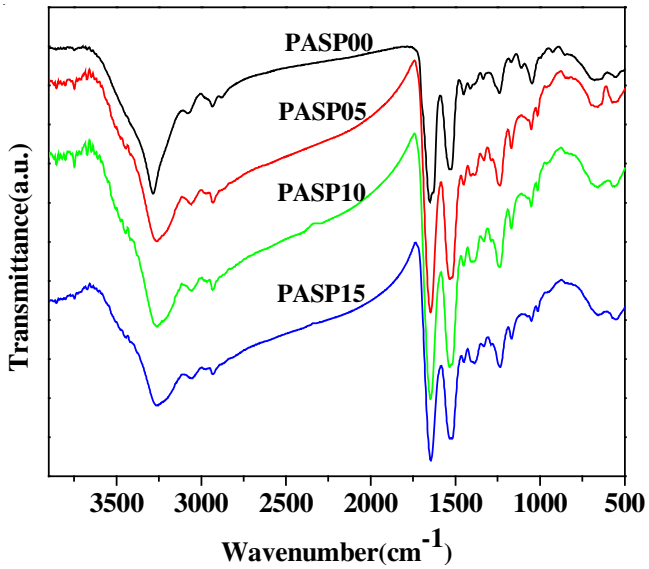

Fig. 2. FT-IR spectra of PASP/SF in different proportions after electro-spinning

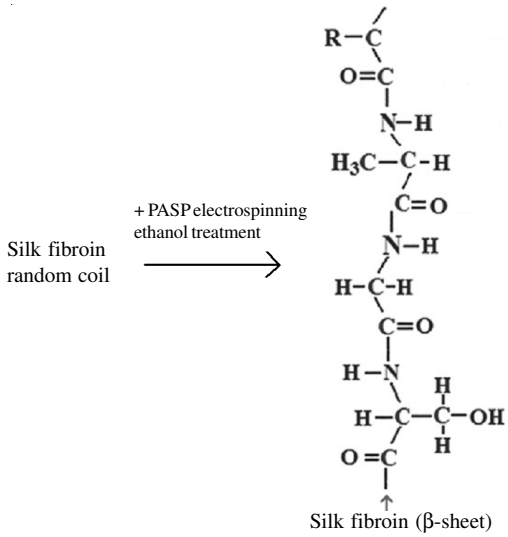

Fig. 3. Schematic diagram of the silk fibroin structure

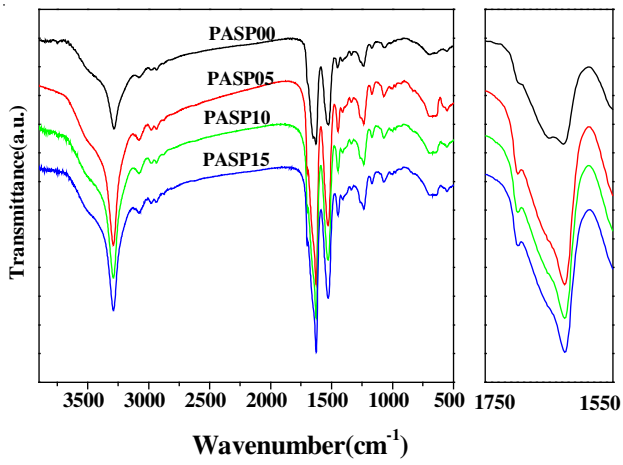

Fig. 4. FT-IR of PASP/SF films in different proportions treated by ethanol

different PASP contents show similar phenomenon. The peaks of PASP05, PASP10 and PASP15 are observed at *ca.* 1627, 1529, 1234 and 3290 cm^{-1} and the shoulder peak *ca.* 3500 cm^{-1}. The peak at *ca.* 3290 cm^{-1} (Fig. 4) is sharper than the band in Figs. 1 and 2. Consequent, these results suggest that the distinct hydrogen bonding states produced by different conformations adopted by the protein chains. In the magnifying FT-IR spectra, the peaks of PASP00 at 1648 cm^{-1} appeared, which indicate that the conformation of the pure SF changed imperfect. And it gets weaker and weaker with the increase of the PASP content. Because the effect of hydrogen bonding gets stronger with more PASP added in. During the treatment of the ethanol solution, the water molecules can easily enter in the free volumes of silk fibroin for its small volume, so that spinning films were swelling. When the spinning films were swelled, films free volumes increased, then the molecular chain have enough space to exercise. And at the same time, large ethanol molecules[21] and PASP[22] molecules can spread into the spinning films to destroy the original hydrogen bond and form the new hydrogen bond. The results lead to make the molecular chain rearrangement and cause the change of the silk fibroin conformation (Fig. 3). In addition, the two strong bands at *ca.* 1625 and 1529 cm^{-1} are sharper than the bands of PASP00. And with the increase of PASP content, the two bands get sharper, thus indicate that the structure of β-sheet is the main structure of the scaffolds and the ethanol is sensitive to the content of PASP. The band at 1650 cm^{-1} (Fig. 1) shifted to 1627 cm^{-1} (Fig. 4). This result suggests that amide I is well changed from silk I to silk II by the ethanol treatment. Also in the magnifying FT-IR spectra, the band of *ca.* 1699 cm^{-1} appears comparing with the spectra in Figs. 1 and 2 and it get stronger with the increase of PASP content. The absorption peak near 1699 cm^{-1} can belong to the turn structure which related to β-sheet conformation[23].

Fig. 5 is the SEM of electro-spinning scaffolds. At the same electro-spinning conditions, pure SF fibers did not reveal smooth, continuous and round as showed in Fig, 5a. And some fibers were even fusion together. The smooth and continuous straight fibers with no beads could be produced from all compositions of the blended solution at the three different contents of PASP (Fig. 5b-d). The applications of electrospun biomaterial fibers have been explored for wound dressing, vascular graft, tissue engineering scaffolds and drug delivery systems. These applications benefit from the high surface area of the fibers and high porosity[13]. A scaffold should be capable of providing mechanical support for cell growth and migration in tissue engineering. Our early work has indicated nano-hydroxyapatite was induced at the surface of SF/PASP blends easily. Evidently, electro-spinning SF/PASP fiber would play a positive role in tissue engineering materials.

Conclusion

Electro-spinning method can change SF structure from random coil and α-helix to more stable structure (β-sheet). And it is also an effective method to prepare porous scaffolds. The treatment of ethanol greatly influenced the molecular conformation of SF. Blending PASP and SF has no obvious effect on the conformation of the SF. While it change greatly with the treatment of electro-spinning and ethanol. However, there are some problems existed in the electro-spinning process, for example: the quantity is little and it is difficult to get nano-size fibers. It is what we need to resolve in next study.

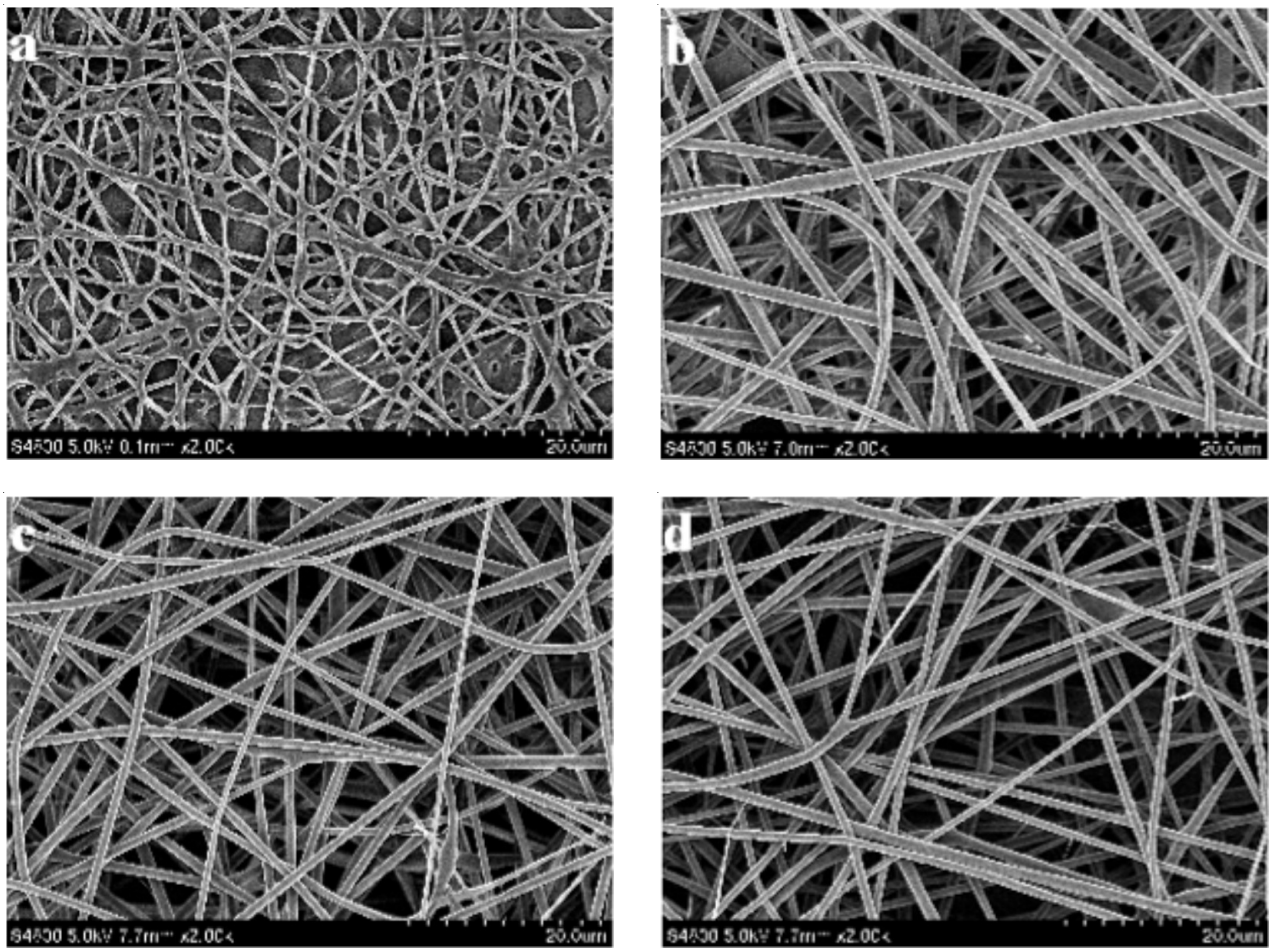

Fig. 5. Scanning electron microscopy of PASP/SF scaffolds (a) PASP00, (b) PASP05, (c) PASP10, (d) PASP15)

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