Supplementary information

Fabrication of high performance silk fibroin fibers via stable jet electrospinning for potential use in anisotropic tissue regeneration

Bingcheng Yi, Huilan Zhang, Zhepao Yu, Huihua Yuan, Xianliu Wang and Yanzhong Zhang

a College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China.
b State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, Donghua University, Shanghai 201620, China.
c Key Lab of Science & Technology of Eco-Textile, Ministry of Education, Donghua University, Shanghai 201620, China.
d School of Life Sciences, Nantong University, Nantong 226019, China.
e China Orthopedic Regenerative Medicine Group (CORMed), Hangzhou 310058, China.

* Corresponding author

Yanzhong Zhang, Ph.D.
Professor of Biomaterials
College of Chemistry, Chemical Engineering & Biotechnology, Donghua University
2999 North Renmin Road
Shanghai 201620, China
Tel/Fax: +86 21 6779 2374
Email: yzzhang@dhu.edu.cn
1. Electrospinning of highly aligned SF fibers

![Figure S1](image)

**Figure S1** (A) Schematic diagram of stable jet electrospinning (SJES) and (B) actual process of the SJES for collecting highly aligned SF-based ultrafine fibers.

2. Spinnability of a series of SF/PEO blend solutions via SJES

SF/PEO fibers were collected in critical stable jet length against the mass ratio of SF and PEO, and the results of their spinnability and diameters were summarized in **Table S1**. The pure SF formic acid solutions were found to be nonelectrospinnable. But upon progressively introducing varied amounts of PEO, the stable jet segment began to grow and its stable jet length was positively correlated to the amount of PEO added.

<table>
<thead>
<tr>
<th>SF/PEO</th>
<th>100:0</th>
<th>96:4</th>
<th>94:6</th>
<th>92:8</th>
<th>90:10</th>
<th>88:12</th>
<th>86:14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinnability</td>
<td>spray</td>
<td>spin</td>
<td>spin</td>
<td>spin</td>
<td>spin</td>
<td>spin</td>
<td>spin</td>
</tr>
<tr>
<td>Collecting distance (cm)</td>
<td>/</td>
<td>5</td>
<td>9</td>
<td>18</td>
<td>20</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Fiber diameter (μm)</td>
<td>/</td>
<td>adhesion</td>
<td>adhesion</td>
<td>adhesion</td>
<td>1.81±0.59</td>
<td>1.20±0.35</td>
<td>2.44±0.55</td>
</tr>
</tbody>
</table>

3. Post-spinning treatments of highly aligned SF fibers

To detect the time frame of water treatment to completely remove PEO, the M-SF/PEO mats were immersed in distilled water for 6, 12, 24, 48, 72, 96 and 120 h with changing water every two hours at room temperature (RT). Then all the fibrous mats were dried in vacuum (RT) for 7 days followed
by weighing via gravimetric method. The results (Figure S2A) show that within the first 12 h of immersion PEO leaching from the M-SF/PEO fibrous mats was the highest rate of extraction. After 24 h massive PEO was leached out of the fibers rapidly and the PEO mass remaining was only 10%. About 96% of PEO was extracted while the fiber mats were treated in water for 48 h, which could be considered that PEO was extracted completely because later on there is merely neglectable variation in PEO extraction. FTIR analysis was conducted to further confirm the fact at molecule level that the PEO was successful extracted from the M-SF/PEO fibers. As shown in Figure S2B, the decreasing absorption peak of PEO at 1101 cm$^{-1}$ (C-O-C) in W-M-SF/PEO fibers further affirms the effectiveness of PEO extraction within 48 h of water treatment. Therefore, during the course of this study, 48 h was selected as the optimal time for water treatment.

To enable water insoluble, as-electrospun SF/PEO fibers with smooth surface (the left SEM image, Figure S2C) were treated in 90% methanol solution, which led to a partial dissolution of the water soluble component of PEO by showing some traces of grooves on the fiber surface (the middle SEM image, Figure S2C). A further water treatment to largely leach out of PEO from the M-SF/PEO fibers made the surface grooves even apparent (the right SEM image, Figure S2C). Whereas the groove direction along the fiber axis suggest the molecular orientation during the SJES process.

Furthermore, to verify whether PEO component of the M-SF/PEO fibers might localize on surfaces of fibers more than cores of fibers, shell-core structured aligned-fibers of PEO (shell)/SF (core) at the mass ratio of 12:88 were fabricated using the SJES approach with 1% PEO (flow rate 1.36 mL/h) and 20% SF solutions (flow rate 0.50 mL/h) at ambient conditions (20-25 °C, 25-30% humidity), and the applied voltage of 7 kV. After the treatment of the as-electrospun PEO (shell)/SF (core) fibers with methanol/water (90:10, v/v), the shell-core structured PEO (shell)/SF (core) mats were immersed in distilled water for 6, 12, 24, 48, 72 and 96 h with changing water every two hours at room temperature. This study (Fig. S3) demonstrated that within the first 6 h of immersion, PEO leaching from the M-SF/PEO fibrous mats was reached to 88.77%. And after 24 h of leaching treatment almost all of the PEO was extracted out of the fibers.
Figure S2 (A) PEO extraction efficiency of M-SF/PEO fibers after leaching treatment at varied period of time in water. (B) FTIR of SF/PEO, M-SF/PEO, W-M-SF/PEO and PEO to ensure a complete extraction of PEO. (C) Morphology of the SF-based fibers before and after subjected to different treatments.

Figure S3 PEO extraction efficiency of coaxial PEO (shell)/SF (core) aligned-fibers after leaching treatment at varied periods of time in distilled water.
4. Mechanical properties of the M-SF/PEO fibers after progressive leaching treatment in water

To examine the influence of PEO extraction on mechanical properties of the M-SF/PEO fibers during PEO extraction, samples after having been soaked in water for 2, 6, 12, 24, and 48 h were immediately tested. As showed in Figure S4, the ultimate tensile strength and the breaking elongation of the M-SF/PEO fibers at wet state both show a declining tendency following the progressive loss of PEO, as leaching out of PEO from the M-SF/PEO fibers undermined the structural integrity by forming internal defects.

Figure S4 (A) Mechanical properties of the M-SF/PEO fibers with different PEO extraction efficiency after being soaked in water for varied periods of time. (B) Statistical results of the mechanical property about tensile strength, strain at break and Young’s modulus for (A).