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Development of Biomimetic Thermoplastic Polyurethane/Fibroin Small-Diameter Vascular Grafts via a Novel Electrospinning Approach

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Abstract

A new electrospinning approach for fabricating vascular grafts with a layered, circumferentially aligned, and micro-wavy fibrous structure similar to natural elastic tissues has been developed. The customized electrospinning collector was able to generate wavy fibers using the dynamic “jump rope” collecting process, which also solved the sample removal problem for mandrel-type collectors. In this study, natural silk fibroin and synthetic thermoplastic polyurethane (TPU) were combined at different weight ratios to produce hybrid small-diameter vascular grafts. The purpose of combining these two materials was to leverage the bioactivity and tunable mechanical properties of these natural and synthetic materials. Results showed that the electrospun fiber morphology was highly influenced by the material compositions and solvents employed. All of the TPU/fibroin hybrid grafts had mechanical properties comparable to natural blood vessels. The circumferentially aligned and wavy biomimetic configuration provided the grafts with a sufficient toe region and the capacity for long-term usage under repeated dilatation and contraction. Cell culture tests with human endothelial cells (EC) also revealed high cell viability and good biocompatibility for these grafts.

Keywords

Vascular graft; thermoplastic polyurethane (TPU); silk fibroin; electrospinning; biomimetic structure

1. Introduction

Since the 1960s, vascular grafts have been used in bypass surgery to replace damaged native blood vessels for treating severe cardio- and peripheral vascular diseases. Autologous vessels such as internal mammary arteries and saphenous veins are regarded as the gold

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standard for bypass grafting. For patients without suitable autologous grafts, synthetic vascular grafts made of polyethylene terephthalate (PET) and expanded polytetrafluoroethylene (PTFE) have been developed as off-the-shelf substitute options. However, for small-diameter synthetic grafts (< 6 mm), thrombus formation in the lumen and internal hyperplasia at the anastomotic site have obstructed vessel patency and restricted their use¹. As a result, the demand for other small-diameter tissue-engineered vascular grafts to overcome these limitations has arisen.

The basic features for a small-diameter, tissue-engineered vascular graft, such as biocompatibility, compatible mechanical properties, and processability, are highly dependent on the matrix materials and the graft structure.² The source of the matrix materials includes cultured cell sheets, decellularized matrices, and natural and synthetic polymers^{3–5}. Among all of them, several types of natural and synthetic polymers have grabbed the attention of researchers due to their intrinsic biological or mechanical properties, accessibility, and manufacturability. More specifically, natural polymers, such as collagen and fibrin, are biologically analogous to the native extracellular matrix (ECM) and can effectively promote tissue remodeling^{6–11}, while synthetic polymers like polyglycolic acid (PGA), polylactic acid (PLA), thermoplastic polyurethane (TPU), polycaprolactone (PCL), and polyurethane (PU) possess tunable mechanical properties, degradation rates, and microstructures^{12–22}. In our previous research, hybrid vascular grafts made of natural silk fibroin and synthetic TPU via the electrospinning technique exhibited the strengths of both natural and synthetic polymers²³. Natural silk fibroin from silkworm (*Bombyx mori*) cocoons possesses the characteristics of low- or non-immunogenicity, relatively slow proteolytic degradation, and low thrombogenicity that make it a reliable material for vascular engineering. TPU is a flexible linear segmented polymer containing soft and hard segments that allow it to behave like an elastomer at normal temperatures, and like a thermoplastic in the molten state. These hybrid grafts have revealed promising cell viability with human endothelial cells (EC) with comparable mechanical properties to natural blood vessels^{24,25}.

For natural blood vessels, their mechanical behaviors are mainly determined by the circumferentially arranged and wavy elastic fibers and collagen fibers in the vessel walls²⁶. The rubber-like elastic fibers contain a core of elastin covered with a sheath of microfibrils that contribute to the elastic behavior of blood vessels, whereas the content of collagen fibers corresponds to the strength/stiffness. Collagen fibers have much less extensibility than elastic fibers, but usually attach to other components loosely and in a folded manner without tension. At a low stretching strain, only elastic fibers elongate while collagen fibers unfold, demonstrating an initial toe region in the stress–strain curve of the blood vessel. As the strain increases, collagen fibers are recruited and stretched, and a steep slope due to their stiffness can be seen^{27,28}. Thus, to design a feasible small-diameter vascular graft, it is advantageous to start from mimicking those aligned and wavy fibrous configurations.

Fibrous structures are beneficial in that they resemble the architecture of natural ECM and vascular tissue, and provide high surface area-to-volume ratios for cell activities. Electrospinning is able to fabricate fibers with diameters ranging from 15 nm to 10 μ m and create high interconnectivity and surface areas^{29–31}. The morphology of electrospun fibers can be controlled to match the desired structure and orientation by altering the working

parameters—including solution properties, processing variables, and ambient conditions—and the types of fiber collector^{32–34}. For instance, to produce tubular grafts composed of circumferentially aligned fibers, one can use a rotating rod to gather the fibers; this method has been employed in certain studies before^{35–42}. However, how to safely remove electrospun samples from a collecting mandrel without causing structural damage has been the main challenge for rod-type collectors. Also, a crimped fibrous architecture is an important factor for providing vascular grafts with similar non-linear stress–strain behavior comparable to native blood vessels. Therefore, a method that can create wavy fibers and solve the removal problem at the same time is required.

In this research, a novel approach has been developed for directly producing a layered, circumferentially aligned, and micro-wavy fibrous structure via an electrospinning technique without post-processing. The customized rotating collector with an assembled mandrel was incorporated to generate TPU/fibroin hybrid vascular grafts with a biomimetic architecture. Depending on the material compositions and processing parameters, the resulting fiber morphologies were different. The mechanism of this new approach will be introduced in the following sections. Also, results of the mechanical characterization and the preliminary biological assays on the produced grafts will be presented and discussed.

2. Materials and Methods

2.1 Device for Fabricating Circumferentially Aligned and Wavy Fibrous Grafts

The customized rotating collector employed here was built with an assembled mandrel, an RS-500 series DC motor (CCL 9015 motor, AndyMark), a DC power supply (QW-MS305D), and an acrylic supporter. The assembled mandrel consisted of a round aluminum tube at the center and several thin satellite cylinders surround the tube. Thin satellite cylinders were arranged equidistantly and fixed to the aluminum tube at the two ends. For this device, the adaptable size of the metal tube ranged from 2 to 5 mm in diameter and the thin satellite cylinders had a diameter of 0.9 mm. The assembled mandrel was installed on the acrylic support with stainless ball bearings at both ends and connected to the DC motor by heat-shrink tubing. The rotation speed (up to 8000 rpm) was controlled by the applied voltage and measured by a digital photo tachometer (DT2234A, CyberTech). Figures 1 (A) and (B) show the setup of the device.

The concept of this design is to make the sample removal process easy and use the swing behavior of the satellite cylinders, like a jump rope, to create a wavy structure along the circumferential direction. When the collector rotates at a sufficient speed, the satellite cylinders form arcs due to the centrifugal force, thus the electrospun fibers are actually collected by a mandrel with a larger diameter. When the collector stops, the thin cylinders move back to their original position, which also draws the electrospun layer to a smaller inner diameter, leading to a wavy wall. The mechanism of wavy-fiber formation is depicted in Figure 1 (C). After the electrospinning process finishes, the thin cylinders can be pulled out first. Then, the tubular electrospun sample will easily slip off the metal tube completely without damage.

2.2 Preparation of Silk Fibroin

Silk fibroin was extracted from the cocoons of *Bombyx mori* silkworms, made into a clear aqueous solution, and dry foamed following the procedure previously described²⁴. Briefly, natural cocoons were boiled in a 0.02 M Na₂CO₃ aqueous solution for 30 min, then rinsed with distilled water and dried out to obtain fibroin fibers. The raw fibroin fibers were dissolved in a 9.3 M LiBr solution and dialyzed against distilled water for 3 days using a cellulose membrane (MWCO 14,000, Sigma-Aldrich). The final concentration of the fibroin aqueous solution was 6 to 8% (w/v). To prepare dry fibroin foam for further usage, the fresh fibroin solution was frozen overnight at -40°C and lyophilized by a freeze dryer (LABCONCO). All of the chemicals used were purchased from Sigma-Aldrich.

2.3 Fabrication of Small-Diameter Vascular Grafts

Medical-grade soft TPU pellets, Tecoflex® SG-80A (Lubrizol), were blended with dry fibroin foam at weight ratios of 2:1, 1:1, and 1:2, and dissolved in hexafluoroisopropanol (HFIP, CovaChem) at 12 w/v%. A TPU solution was also prepared at 10 w/v% by dissolving the pellets in a mixed solvent of chloroform and dimethylformamide (CF/DMF = 3/1 v/v, Sigma-Aldrich). All of the mixtures were stirred at room temperature for 12 hours before electrospinning. The conductivities of the TPU/fibroin solutions were measured by a portable pH/conductivity meter (AP85, Fisher Scientific). In the electrospinning trials, the solution was infused through a 10 mL syringe and tubing by a syringe pump (Harvard Apparatus 11 Plus) to an 18 gauge metal needle. The flow rate was 0.6 mL/h, the applied voltage was 14 to 15 kV, the needle-collector distance was 20 cm, and the humidity was maintained at 35 to 40%. The rotating speed of the customized collector was 5000 rpm. Each trial was conducted at room temperature for 5 hours. At the end, electrospun samples containing fibroin were placed in a vacuum desiccator with methanol for 48 hours to induce the conformational transition of fibroin to make it water-insoluble.

2.4 Characterization

The microstructure and morphology of the electrospun TPU/fibroin and TPU grafts were observed by scanning electron microscopy (JCM-5000 NeoScope, JEOL) with an accelerating voltage of 10 kV. The samples were gold sputter coated for 45 seconds before imaging. Fiber diameters were measured using the image processing software Image-Pro Plus (Media Cybernetics).

Circumferential tensile tests were conducted for TPU/fibroin grafts on an Instron 5967 testing frame with a 30 kN load cell and Biopuls pneumatic clamps ($n = 6$ for each group). Two L-shape grips were used to hold and stretch the tubular specimen in the transverse direction at a crosshead speed of 5 mm/min until it broke. Specimens for the tests were 3 mm in length, with a 3.5 mm inner diameter and measured wall thickness. A micrometer was used to measure the thickness before every trial. Circumferential cyclic responses were also measured to observe the repeatability of these hybrid grafts and to compare their behavior to natural blood vessels. Each tubular specimen was 5 mm long and stretched in the transverse direction at a constant elongation rate of 5 mm/min to 20% strain and then released to 0% strain for 300 cycles.

Suture retention tests were conducted according to ISO 7198. Each specimen was 2 cm in length. A 5-0 suture with a FS-2 needle (PROLENE 8661G, Ethicon) was punctured perpendicularly through the specimen at a location 3 mm from the edge. The specimen and the suture were then fixed on the lower and upper clamps of the tensile tester, respectively. The suture was pulled at a constant rate of 120 mm/min until the specimen was completely torn. The maximum force was recorded as the suture retention strength. For all mechanical characterization tests, all samples were tested in wet conditions.

Surface wettability on the inner surface of electrospun TPU/fibroin and TPU grafts was assessed on a Dataphysics OCA 15 optical water contact angle (WCA) measuring system using the sessile drop method at room temperature. The droplet was 4 μ L of deionized water. Three samples were tested for each group and images at 0, 15, and 60 seconds were recorded for measuring the contact angle.

2.5 Cell Culture and Biocompatibility

A preliminary assay of cell–matrix interactions was conducted by seeding human endothelial cells (ECs) on selected electrospun TPU/fibroin and TPU grafts. Human ECs derived from embryonic stem cells were prepared for this cell viability testing. The differentiated endothelial cells were cultured in E7V media for another three days before seeding on the samples. Cells were digested with Accutase for 3 min and seeded vertically on a straight tube at a seeding density of 5×10^4 for live/dead assay and MTS assay to evaluate the viability and proliferation of ECs on the grafts. Live/dead assay viability/cytotoxicity kit (Life Technologies) was used to target live cells with green fluorescence (calcein-AM) and dead cells with red fluorescence (EthD-1). Stained samples were imaged using a Nikon Ti-E confocal microscope at the day 14 time point. Cell proliferation was evaluated using MTS assay after culturing for 1, 7, and 14 days using a CellTiter 96 Aqueous One Solution kit (Promega Life Sciences). Cells were treated with media containing 20% MTS solution and incubated for 1 h. Then, 100 μ L of spent media were transferred into a clear 96-well plate. The absorbance of the plates at a wavelength of 450 nm was read with a Glomax-Multi+Multiplate Reader (Promega). The subsequent number of cells was determined relative to the negative control.

To investigate whether the endothelial cells were able to cover the inner surface of the grafts, a special cell seeding method was proposed. Briefly, the cell suspension (1×10^7 cells/mL) was injected into the tubular grafts using a 200 μ L pipet. The cell-seeded samples were carefully transferred on a polydimethylsiloxane (PDMS) V-shaped notch on a cell plate filled with E7V media. The plates were then transferred into a cell culture incubator and cultured for two hours. Two hours later, cell seeding was repeated, and the samples were turned 180 degrees to make sure that the cells were seeded on all inner surfaces. Cell morphologies were observed with the same confocal microscope at day 5 of culturing.

3. Results and Discussion

3.1 Structure and Morphology

The rotating assembled mandrel used the change in collector diameter during and after electrospinning to create a circumferentially aligned and wavy fibrous structure that can mimic the configuration and mechanical behaviors of natural blood vessels. Figure 2 shows the cross-section and inner surface of a graft made of TPU/fibroin (1:1) at different magnifications. The inner diameter of this hybrid graft was about 5 mm, and a wavy inner surface and a smooth outer surface could be clearly seen in Figure 2 (A) as depicted by the profile of the inner surface. At a higher magnification, the wall of the graft was found to be composed of a thick inner layer and a thin outer layer (Figure 2 (B)). Upon further inspection, it was discovered that the crest part of the inner surface was formed by the accumulation of crimped fibers that became several wavy layers (Figure 2 (C)). The inner surface of the grafts presented a continuous wavy-flat alternating structure along the circumferential direction, as shown in Figure 2 (D). The flat regions were the areas that directly contacted the satellite cylinders during processing.

The mechanism of creating wavy inner layers and smooth skin layers is depicted in Figure 3. When the electrospinning process begins, the collector starts rotating, and the satellite cylinders swing away from the central axis due to the centrifugal force applied to them. In the early stage, the electrospun fibers are collected by a mandrel with a large diameter. As the process progresses, more and more fibers deposit on the rotating collector and the sample thickness increases. For polymer materials that shrink at room temperature, such as TPU, the shrinkage of collected fibers starts to resist the centrifugal force from the rotation and push the satellite cylinders back to the metal tube. At this stage, the fibers crimp and form a wavy structure between the satellite cylinders. When the shrinkage of fibers completely overcomes the centrifugal force, the satellite cylinders are tightly bound to the metal tube and a smooth skin layer is constructed by the following fibers.

Depending on the material compositions, the resulting fiber morphology is different. This fact can be observed by comparing the fibers in the wavy region and the outer surface of various grafts. Figure 4 presents the images of wavy fibers and fiber diameter distribution from the grafts made of different TPU/fibroin blends and pure TPU. It can be seen that the waviness of the fibers was most distinguished on the TPU/fibroin (2:1) sample, which was due to adequate shrinkage from the TPU. With decreasing TPU content, the fibers became less wavy. In the TPU/fibroin (1:2) sample, waves of fibers were low and could only be seen at high magnification. However, for a graft completely made of pure TPU, no wavy structure was found since the shrinkage of the fibers was too strong and bound the satellite cylinders to the original position within 30 minutes of spinning. In this case, the fibers had no chance to become crimped and further accumulate to form wavy layers.

The average fiber diameter decreased when the content of fibroin increased in the hybrid grafts. In particular, the average fiber diameter of TPU/fibroin grafts with polymer ratios of 2:1, 1:1, and 1:2 were $1.61 \pm 0.74 \mu\text{m}$, $1.31 \pm 0.62 \mu\text{m}$, and $0.74 \pm 0.58 \mu\text{m}$, respectively. Pure TPU grafts were made with different solvents; therefore, they did not follow the trend. Their average diameter was $0.98 \pm 0.35 \mu\text{m}$. According to previous studies, the decrement of

fiber size could have been caused by the increase of solution conductivity and the reduction of viscosity when a single solvent was used^{43–46}. When the solution conductivity increased, more charges were carried by the solution and enhanced the elongational force applied to the electrospinning jet, leading to the formation of thinner fibers²⁹. Measurements showed that the conductivities of the TPU/fibroin solutions were 1.45, 3.09, and 11.6 $\mu\text{S}\cdot\text{cm}^{-1}$, which increased with the content of fibroin. The solution viscosity also decreased visibly when the content of TPU decreased. Moreover, the fibers with relatively large fiber diameters were found to align better than those with smaller diameters. Pure TPU fibers showed the worst alignment due to the slow evaporation of solvent (CF/DMF).

Furthermore, from the images of the graft's outer surfaces, it was obvious that the selection of solvent and the addition of fibroin could influence the occurrence of fiber conglutination (Figure 5). Conglutination happens when the polymer fibers are collected before the solvent completely evaporates. The strong attachment between overlapped fibers enhances the stiffness of the electrospun matrix^{47,48}. In this study, HFIP was used as the solvent for TPU/fibroin blends, while CF/DMF co-solvent was applied for pure TPU. Comparing these two solvents, HFIP was more volatile than the CF/DMF blend due to its relatively low boiling point and high vapor pressure (Table 1). The difference of volatility was also observed during the electrospinning trials. Since HFIP had a faster evaporation rate than CF/DMF, less conglutination formed in the TPU/fibroin hybrid grafts than the TPU one. Besides the rapid evaporation, the higher conductivity of the TPU/fibroin (1:2) solution also induced stronger electric repulsion between the deposited and incoming fibers. The repulsion suppressed the attachment between electrospun fibers, thus the TPU/fibroin (1:2) sample had many fewer conglutinated sites and more separate fiber distribution structure than the TPU/fibroin (2:1) and TPU samples.

3.2 Mechanical Properties and Surface Wettability

TPU/fibroin hybrid grafts composed of circumferentially aligned and wavy fibers were directly fabricated by the newly-developed collector via the electrospinning method. The purpose of the wavy fibrous structure is to mimic the non-linear stress–strain behavior of natural blood vessels, offering compatible mechanical characteristics to vascular grafts. Matching the mechanical properties of natural tissues is crucial for vascular grafts since turbulent blood flow and stresses exerted by mismatches can lead to thrombosis and graft obstruction. It is expected that the uncrimping of wavy fibers can resemble the initial toe region, and the intrinsic material properties of TPU and fibroin can provide the required elasticity and strength for the linear stress–strain behavior as natural elastic fibers in blood vessel walls. To evaluate the performance of these hybrid grafts, a series of mechanical tests were conducted.

Figure 6 and Table 2 present the results of circumferential tensile tests, including representative stress–strain curves, toe regions, ultimate tensile strengths, strains-at-break, and Young's moduli for all TPU/fibroin hybrid grafts. The toe region was due to the straightening of the crimped elastic fibers. The experimental toe regions of human coronary arteries and saphenous veins are between 18 and 38%⁴⁹, and 23 and 36%⁵⁰, respectively. Compared to that, all of the hybrid grafts had longer toe regions that fell between 29 and

53%, which indicated their ability to expand and contract without structural damage under a dynamic force. The shorter toe region of the TPU/fibroin (1:2) graft was due to the lesser wavy structure as compared to the other two combinations. Owing to the intrinsically low elasticity of fibroin, the TPU/fibroin (1:2) graft also possessed the lowest extensibility and ultimate strength among the three, but was still fairly accommodating for vascular applications. From other studies, the experimental circumferential ultimate strength and strain of natural human blood vessels varied from 0.8 to 3.3 MPa and 49 to 105%, respectively^{50–53}. It was anticipated that the TPU/fibroin (2:1) graft would exhibit the lowest Young's modulus and highest strain-at-break due to having the highest TPU content and the waviest structure among the three blends. However, because of the conglutination between the electrospun fibers, the structure of the TPU/fibroin (2:1) graft was stiffer and the extension of fibers was hindered, which greatly increased the Young's modulus and reduced the ultimate strain.

The cyclic circumferential tensile properties of the three TPU/fibroin hybrid grafts were measured to observe their repeatability and to compare their behavior to natural blood vessels. Figure 7 shows the recorded stress–strain curves of the 1st, 100th, 200th, and 300th cycles for each group, where the scatter data points represent the experimental stress–strain range of human coronary arteries from other research⁴⁹. Similar to natural soft tissues, the first cycle of the grafts was for stress softening and directional stretching and loosening pulled the fibers apart and reoriented them, making the stress–strain behavior different from the rest of cycles⁵⁴. According to the experimental results, the circumferential cyclic behaviors of all of the TPU/fibroin grafts were in good agreement with that of natural human coronary arteries. The wavy structure created by the assembled rotating collector effectively increased the toe region and showed no structural damage during repeated loadings, providing the grafts with the capacity for long-term usage. Among the three blends, TPU/fibroin (1:1) had the most comparable properties to natural arteries and was selected as the substrate for cell culture tests.

Suture retention strength is one of the indicators for representing the anastomotic strength of conventional vascular grafts. Grafts with sufficient suture retention strength are required for surgeons to conduct successful implantations^{55,56}. From the tests, it was clear that TPU/fibroin (1:2) had the lowest strength, but the difference between the TPU/fibroin (2:1) and (1:1) grafts was not significant (Figure 7). For grafts with higher TPU content, the intrinsic material elasticity of TPU, and the conglutination between fibers, could have retarded the cracking of the pin holes, thereby improving the final suture retention strength. Overall, the force required to pull the suture through the graft was higher when the TPU content was 50% or greater of the material composition.

Surface wettability, namely the water contact angle (WCA) on the surface, is an important factor reflecting the hydrophilicity and hydrophobicity of a graft and determines the protein absorption and cell adhesion. To investigate the surface wettability on the inner surface of each electrospun graft, samples of TPU and the three TPU/fibroin hybrid grafts were prepared for the water contact angle test. Captured images of all of the samples at 0, 15, and 60 seconds are shown in Figure 8 and Table 3. From the images, it is clear that the hydrophilicity of the electrospun grafts was enhanced with the incorporation of fibroin.

Comparing TPU and TPU/fibroin (1:2) samples, the water contact angle at 0 seconds dropped from $139.7 \pm 0.5^\circ$ to $122.6 \pm 2.0^\circ$. Also, the water absorption was faster in the samples containing more fibroin. The change of angle from 0 to 60 seconds for the TPU/fibroin (2:1) sample was about 4° , while it was 34° for the TPU/fibroin (1:2) sample. Like most synthetic polymers, TPU is hydrophobic. By blending fibroin and TPU, the resulting moderate surface wettability could be beneficial for cell adhesion on the grafts.

3.3 Cell Culture Results

Recall that among the three TPU/fibroin samples, TPU/fibroin (1:1) had the most comparable properties to natural arteries. In this study, electrospun TPU and TPU/fibroin (1:1) hybrid grafts were selected for cell viability assays with human endothelial cells (EC) to verify the biocompatibility of the applied materials and to inspect the effects of fibroin on cell activity. As shown in Figure 9 (A), after 14 days of culture, the cells attached on the tubular grafts were mostly live cells, as indicated by the green dots. There were almost no dead cells visible from the images for both TPU and TPU/fibroin (1:1) grafts suggesting high cell viability. Moreover, it was found that cells grew on both the inner and outer surfaces of the TPU/fibroin (1:1) graft, while they mostly only grew on the inner surface of the TPU graft. The cell proliferation data (Figure 9 (B)) showed that the TPU/fibroin (1:1) graft had a significantly higher cell population than the TPU graft at all time points indicating that the addition of silk fibroin greatly enhanced the biocompatibility of the graft. When cells were seeded twice on the grafts horizontally at a high seeding density, full coverage of the inner surface of the vascular grafts was achieved, even after 5 days of culturing, as shown in Figure 10. From the image, one can see that cells were connected to each other, thereby forming a continuous membrane on both grafts. From the number of nuclei, it was clear that the TPU/fibroin hybrid graft had a much higher cell population than the pure TPU graft, which is in agreement with the vertical culture results and also suggests that the TPU/fibroin hybrid graft could accommodate more cells and form a denser endothelia membrane than the TPU graft. Therefore, these results revealed the applicability of TPU and fibroin as materials for vascular grafts and the introduction of fibroin greatly enhanced the biocompatibility.

4. Conclusion

Small-diameter vascular grafts made of natural silk fibroin and TPU were produced by using a novel rotating collector in an electrospinning process. The assembled mandrel was composed of a metal tube at the center and several satellite cylinders that surrounded it. The dynamic “jump rope” effect of the rotating collector created a continuous wavy-flat alternating fibrous structure along the circumferential direction that provided the graft with more extensibility than a regularly aligned one. When the electrospinning process finished, the satellite cylinders of the assembled mandrel could be pulled out first, then the whole collected tubular sample would slip off the mandrel easily without damage. For TPU/fibroin and TPU grafts, the material compositions and solvent selection affected both the aligned and the wavy structure of the electrospun fibers. The waviness of the crimped fibers in the TPU/fibroin hybrid grafts increased with the TPU content due to greater shrinkage of TPU molecules. At the same time, TPU-rich fibers in the outer surface and the flat region tended

to have conglutinations that stiffened the matrices. Pure TPU grafts had no wavy structures since TPU is a highly-shrunk and elastic material. All of the TPU/fibroin hybrid grafts displayed similar mechanical properties to natural blood vessels in the circumferential direction. Water contact angle (WCA) tests revealed that TPU was hydrophobic and fibroin was hydrophilic. By blending these two materials, grafts with adequate hydrophilicity can be fabricated, which is beneficial for cell–matrix activity. On the TPU/fibroin hybrid graft, cell populations were much higher than on the pure TPU graft, which indicated that the addition of fibroin significantly enhanced cell proliferation.

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Figure 1.

(A) Schematic of the electrospinning apparatus with a customized rotating collector. (B) Rotating collector and assembled mandrel. The assembled mandrel is comprised of a hollow metal tube at the center and several surrounding satellite cylinders. It is mounted on an acrylic support with stainless ball bearings at both ends. (C) The mechanism of how the circumferentially aligned and wavy fibers formed on the rotating collector with the assembled mandrel.

Figure 2.

(A) TPU/fibroin (1:1) graft with a wavy inner surface and a smooth outer surface. (B) The wall of the graft was composed of a thick layer with various thicknesses and a thin skin layer. (C) The crest of the inner layer (the squared region in (B)) was formed by the accumulation of crimped fibers. (D) Fiber morphology of the inner surface.

Figure 3.
Formation of a wavy inner layer and a smooth outer skin layer.

Figure 4. Fiber morphology of the wavy region from grafts made of various material compositions.

Figure 5.
Fiber morphology of the outer surface. The TPU/fibroin (1:2) graft had the least conglutination.

Figure 6. Results of circumferential tensile tests for electrospun TPU/fibroin hybrid grafts, including representative stress–strain curves, toe regions, ultimate tensile strengths, strains-at-break, and Young's moduli.

Figure 7. Cyclic response and suture retention strength of TPU/fibroin hybrid grafts. Scatter data points represent the lower and upper bounds of human coronary arteries from experiments⁴⁹.

Figure 8. Results of water contact angle tests for various grafts at 0 (top), 15 (middle), and 60 (bottom) seconds.

Figure 9. Human endothelial cell culture results on TPU and TPU/fibroin (1:1) grafts at low seeding densities with vertical seeding. (A) Fluorescence images of live/dead assay at day 14. (B) Cell proliferation results from MTS assay at days 1, 7, and 14.

Figure 10.

Endothelial cell culture results on TPU and TPU/fibroin (1:1) grafts at high seeding densities with horizontal seeding. Red and green fluorescence indicate CD144 cells and nuclei.

Depending on the intensity of the fluorescence, the coverage area and population of cells on the two samples can be compared.

Table 1

Properties of applied solvents.

Solvent	Boiling point (°C)	Vapor pressure at 20°C (kPa)
HFIP	58	16
CF	61	21
DMF	153	0.36

Table 2

Mechanical properties of TPU/fibroin hybrid grafts. Six specimens were tested for each group.

	TPU/fibroin (2:1)	TPU/fibroin (1:1)	TPU/fibroin (1:2)	Human Blood Vessels ⁴⁹⁻⁵³
Toe region (%, mm/mm)	42.9±5.1	45.4±6.8	38.3±9.7	18–38
Ultimate strength (MPa)	1.10±0.23	1.61±0.37	0.77±0.11	0.8–3.3
Strain at break (%, mm/mm)	123±18.8	166±27.6	109±6.39	49–105
Young's Modulus (MPa)	1.82±0.12	1.19±0.31	1.42±0.09	1.2–9.7

Table 3

Average values of water contact angles for all the samples.

Time (s)	TPU	TPU/Fibroin (2:1)	TPU/Fibroin (1:1)	TPU/Fibroin (1:2)
0	139.7 ± 0.5	133.0 ± 2.2	132.2 ± 0.6	122.6 ± 2.0
15	138.9 ± 0.8	132.1 ± 2.6	130.8 ± 1.2	114.5 ± 0.9
60	135.6 ± 1.2	128.7 ± 1.7	114.8 ± 1.9	88.9 ± 1.1