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Preparation of Multilayer Biodegradable Nanofibers by Triaxial Electrospinning

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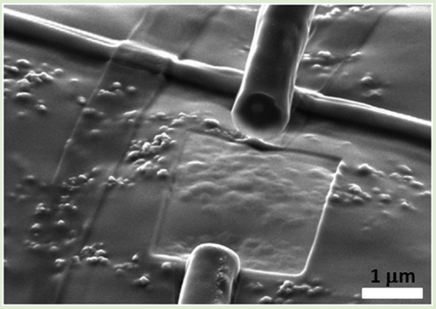
ABSTRACT: A biodegradable, multilayer nanofiber structure has been successfully developed by using a self-designed and fabricated triaxial electrospinning system using gelatin as the sheath and core layers and poly(ε-caprolactone) (PCL) as the middle layer. The triaxial structure was investigated by confocal fluorescence microscopy (CFM) and focused ion beam and field-emission scanning electron microscopy (FIB-FESEM). The ability to fabricate the multilayered nanofibers efficiently with different biodegradable polymers will enable this triaxial electrospinning technique to have wider applications in biotechnology.



Electrospinning is well-known as a simple and scalable technique to develop polymer solutions or melts into nanofibers under an electric field.[1] Coaxial electrospinning is a modification of traditional electrospinning, and in a typical coaxial electrospinning setup, the spinneret consists of two concentric needles.[2] Core and shell solutions are delivered to different needles by individual pumps and finally meet at the tip of a concentric spinneret. Coaxial eletrospinning has attracted much attention as an efficient way to fabricate hollow fibers,[3−5] to incorporate nanoparticles,[6] or to engineer core−sheath structured[7,8] nanofibers, which have applications in catalysis,[4] filters,[9] hydrogen storage,[10] and especially biomedical applications.[11,12] Many studies have also been reported on the preparation of core−sheath biodegradable nanofibers with application in tissue engineering,[13] drug delivery,[11,14] wound healing,[8] etc. Zhang et al.[15]studied the mechanical properties and cell proliferation of collagen-coated poly(ε-caprolactone) (PCL) nanofibers developed by coaxial electrospinning. Wang et al.[11]prepared core/shell nanfibers with two biodegradable polymers, poly(DL-lactic acid) (PDLLA) and poly(3-hydroxy butyrate) (PHB), and studied release rates of an incorporated dimethyloxalylglycine (DMOG) drug with different shell thicknesses. Among these studies, the three main objectives have been: (1) increasing the mechanical properties of the fiber by using a stronger core; (2) incorporation of drugs into the core; and (3) increasing the biocompatibility of fibers by using a more natural polymer as the sheath. Although most coaxial electrospun fibers could satisfy one or two of the issues mentioned, it is difficult to realize all three objectives with a two-layer structure. Our motivation for this study is to fabricate multilayer structured nanofibers through triaxial electrospinning and study their application to biomedical devices. Triaxial electrospinning uses a spinneret with three concentric needles as illustrated schematically in Figure 1. Three solutions delivered by different pumps meet at the tip of the spinneret. The solution deforms into a Taylor cone under an electrostatic field, and a triaxial jet emerges when the electrostatic force overcomes the surface tension of the solution. The jet undergoes a bending instability, whipping motion, solvent evaporation, and finally deposition on the collector as dry fibers.[1,16]

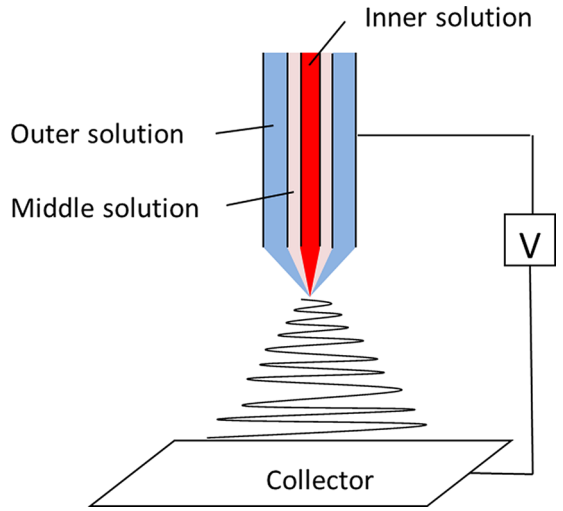


Figure 1. Schematic diagram of the triaxial electrospinning setup.

Joo et al.[17] reported the fabrication of triaxial electrospun fibers with silica as the shell and core layers and with a self-assembling polymeric material as the intermediate layer. On the other hand, Chen et al.[18]developed nanowire-in-microtube structured nanofibers through triaxial electrospinning. However, our work is the first report of triaxial electrospinning of biocompatible polymeric nanofibers. In our study, we have

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fabricated gelatin/PCL/gelatin multilayer structured nanofibers via triaxial electrospinning. Gelatin is a natural biodegradable polymer that has very good biocompatibility for cells and allows for cell adhesion and proliferation.[19] The primary limitation of gelatin is the mechanical strength. PCL is a synthetic biodegradable polymer with very good mechanical properties and low degradation. However, PCL is not as attractive as natural polymers for cells since it is hydrophobic.[20] Core−shell structured gelatin/PCL nanofibers combine the advantages of PCL and gelatin and avoid their limitations.[7] In addition, gelatin serves well as a drug carrier,[21] and electrospun nanofibers with gelatin as the core would have potential for controlled drug release. Furthermore, the coaxial electrospinning of PCL and gelatin[7,12] has been reported as well as electrospinning of pure gelatin[22]and pure PCL.[23] All of these studies provide a basis for our study on triaxial electrospinning of gelatin/PCL/gelatin nanofibers.

The morphology of triaxial electrospun gelatin/PCL/gelatin nanofibers is shown by the scanning electron microscopy (SEM) image in Figure 2. The diameters of the fibers are approximately 1000 nm.

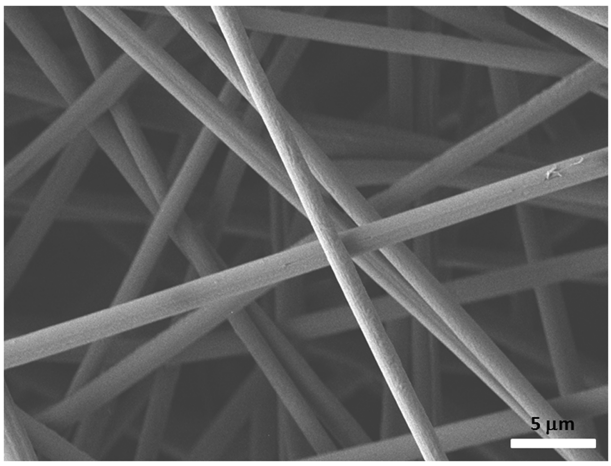

Figure 2. SEM image of triaxial electrospun gelatin/PCL/gelatin fibers.

For a coaxial core−sheath structure, the commonly used characterization methods are transmission electron microscopy (TEM),[2,6,7] cross-section TEM,[17] and cross-section SEM.[3−5] However, TEM is not the preferred choice for analyzing triaxial multilayered structures due to the low contrast of electron densities between the polymer phases and the overlapping of layers. In addition, the deformation of polymer fibers during the microtoming process limits the application of cross-section TEM and cross-section SEM since artifacts of the preparation process may occur. In our study, two methods, confocal fluorescence microscopy (CFM) and field-emission scanning electron microscopy (FIB-FESEM), were employed to investigate the triaxial structure.

For CFM measurements, a fluorescent dye, rhodamine B, was added into both shell and core gelatin solutions before electrospinning. Figure 3 shows the high-resolution CFM image of a triaxial electrospun nanofiber. The two outermost, thinner fluorescent lines, although occasionally discontinuous, indicate the presence of the shell, while the central, thicker fluorescent line indicates the presence of the gelatin core. The non-fluorescent area between the narrow and thicker red lines is

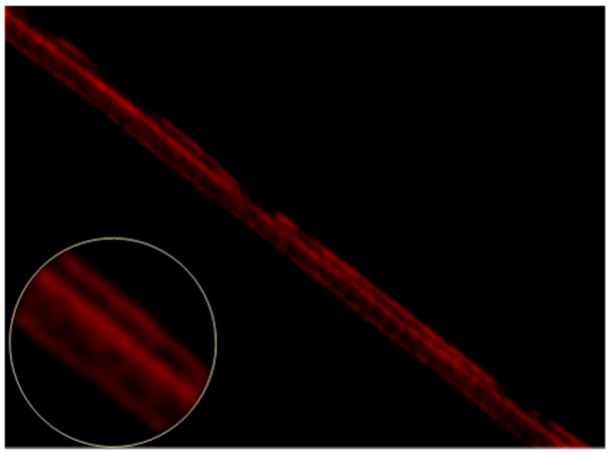

Figure 3. Confocal fluorescence microscopy image of a triaxial electrospun nanofiber.

indicative of the undyed PCL middle layer, which does not fluoresce.

To visually observe the multilayer structure, the fibers were then analyzed using a FIB-SEM. An ion beam probe was used to cut the fiber and remove a part of the fiber through milling, and then the cross section of the fiber was imaged under the SEM (Figure 4). The contrast of different layers due to the difference in secondary electron densities between polymer phases clearly demonstrates the existence of a triaxial structure. As can be seen in both the left and right images of cut fiber cross sections, there is a light shaded gelatin core surrounded by a dark (PCL) intermediate layer, which, in turn, is then surrounded by a thin outer sheath of gelatin. The FIB-FESEM image also indicates the specific thickness of each layer, which is 130 nm for the sheath, 240 nm for the intermediate layer, and 230 nm for the core layer. The CFM and FIB-SEM images unequivocally prove the existence of the three concentric polymer layered nanofibers produced by our unique triaxial electrospinning apparatus.

In summary, we have shown multilayer structured biodegradable fibers fabricated through triaxial electrospinning. Compared with coaxial electrospun core/shell nanofibers, the addition of the third layer provides possibilities for the development and improvement of functional tissue scaffolds for drug delivery and wound healing. By using gelatin as both the shell and core layer and PCL as the middle layer, we attempted to provide a functional triaxial scaffold for tissue engineering. Here, the advantages of this design could be: (1) the biocompatible sheath allows cells to adhere and proliferate; (2) the middle layer provides adequate strength to support the developing tissue; (3) growth factors or drugs can be incorporated into the core and released through diffusion and degradation. In addition these triaxial nanofibers could be used for controlled drug release by incorporating different drugs into each layer.

■ EXPERIMENTAL SECTION

Gelatin was obtained from Eastman Kodak Co., NY. PCL (Mn = 45 000), and 2,2,2-trifluoroethanol (TFE) was purchased from Sigma-Aldrich. The shell and core solutions were composed of 17 wt % and 10 wt % gelatin, respectively, in 80/20 w/w TFE/deionizer water. The middle layer solution was 11 wt % PCL in TFE. Three solutions were loaded independently into the triaxial concentric nozzle. Three pumps were applied to keep the flow rate at approximately 1.0, 0.4, and 0.15 ml/h, from shell to core. An aluminum foil covered cardboard sheet

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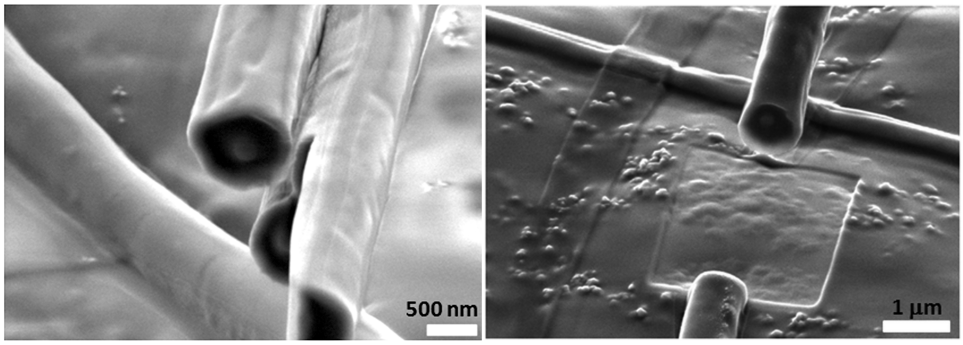

Figure 4. FIB-FESEM images of triaxial electrospun nanofibers.

was used as the collector with a high voltage applied between the nozzle and the collector. Dry fibers accumulated with random orientation on the collector plate in the form of a nonwoven mat.

The morphology of the electrospun fibers was observed by JEOL JSE 7400F SEM. The CFM image was taken with a Carl Zeiss LSM 710. In addition, a FIB-FESEM (Zeiss Auriga 60) was utilized to investigate the interior structure of the fibers. The milling current and imaging current in the FIB were 50 pA, while the accelerating voltage in the SEM was 3 KV.

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Notes

The authors declare no competing financial interest.

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