Co-Electrospun Nanofiber Fabrics of Poly(L-lactide-co-ε-caprolactone) with Type I Collagen or Heparin

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Introduction: Recently, the scaffold fabrication and design of submicron- to nanoscale structural architectures, which geometrically or topologically mimic the native state of ECM, have received much attention in tissue Engineering. Electrospinning, which is a fiber spinning technique driven by a high-voltage electrostatic field, has recently attracted considerable attention in medical applications, and produces three-dimensional scaffolds with a well-defined nano- to micro-architecture including controlled pore size, fiber diameter, and topography. Poly(L-lactide-co-ε-caprolactone) (PLCL), is a biodegradable elastomer. A biochemically and functionally designed PLCL-based scaffold, which is combined with a nanofabric structure, and which has elastomeric properties, biodegradability, and cell adhesion and anticoagulation activity, may enable the engineering of bioactive and bioinducible vascular tissues. To this end, we determined that enhanced cell adhesivity was generated by co-electrospinning of PLCL and type I collagen, and that potent anticoagulation activity was achieved by co-electrospinning of PLCL with tri-*n*-butylamine salt of heparin (heparin-TBA). These bioactive biomacromolecule loaded co-electrospun fabrics were, to our best knowledge, the first to be reported in biomedical applications.

Method: ***Copolymerization.*** The equimolar copolymer of L-lactide and ε-caprolactone was synthesized by ring-opening polymerization in the presence of $Sn(Oct)_2$ at 150°C for 24 h.

Heparin-TBA preparation. An aqueous solution of heparin sodium salt (Sigma, 180 U/mg), was passed ion-exchange column (Dowex 50W). To the solution, a methanol solution of TBA was added, and the mixture was stirred for 2 h (Scheme)

Electrospinning. For co-electrospinning, PLCL with collagen or heparin-TBA was dissolved in a hexafluoro isopropanol (HFIP, Aldrich) at a final concentration of 3 wt.%. The mixing ratio (%) of collagen or heparin-TBA to the copolymer was 0, 5, 10, 30, 50, 70, and 100 wt.%, and heparin-TBA was 1, 5, and 10 wt.%. The mixing solution was delivered at a flow rate (5 ml/h) with an air gap between metal collector and needle tip (20 cm) at a driving voltage (25 kV).

Microscopic observations. Co-electrospun collagen or heparin-TBA-blended PLCL fabrics were observed under a transmission electron microscope (TEM, Hitachi).

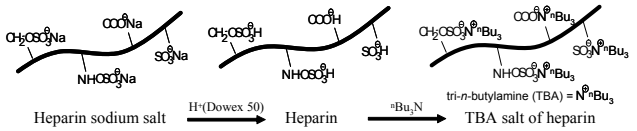
Tensile test. The stress-strain curves of co-electrospun PLCL fabrics were characterized at a stretching speed of 0.5 mm/s.

Cell culture. Human umbilical vein endothelial cells (HUVECs) were cultured on the co-electrospun fabrics, and the number of cells was determined using a hematocytometer.

Release study of heparin-TBA. The amount of heparin-TBA released from the fabric was determined by a toluidine blue method using a visible spectrophotometer at 631 nm.

Results and Discussion: The number-average molecular weight (Mn) and polydispersity (Mw/Mn) of PLCL, determined by GPC, were 2.6 x 10^5 and 1.8, respectively. Rapid full-monolayer formation of endothelial cell (complete endothelialization) and potent anti-coagulation are essential for replacement of small-diameter diseased vessels. To this end, PLCL copolymer blended with different mixing ratio of collagen to PLCL. All the co-electrospun fabrics produced consisted of nano-scale fibers with a mean diameter ranging from approximately 120 nm to 520 nm. An increase in the collagen ratio resulted in a continuous decrease in the mean diameter of fibers, probably due to the electrical properties of collagen, which consists of electrically charged amino acids. Collagen molecules in a fine structure of co-electrospun fibers, determined by TEM, at low concentrations of collagen, appeared to aggregate to form spherical domains in the PLCL continuous matrix phase (Fig. 1-A), whereas at high concentration, high-intensity localization of collagen in the peripheral region of the continuous matrix phase was observed (Fig 1-B). Mechanical strength was significantly decreased with increasing collagen content: Young's modulus of PLCL/50 wt.%-collagen fabric in water was approximately one-hundredth that of PLCL fabric. Regarding the behavior of cells on the fabrics, higher cell adhesion and proliferation of HUVECs were noted on both fabrics containing a small amount of blended collagen (5 and 10%), otherwise cells could not spread and the number of cells was decreased on blended PLCL fabrics containing a large amount of collagen (30 and 50%), due to shrinkage during the 5-day culture caused by the decreasing mechanical strength of these fabrics. Highly ionic-charged heparin does not dissolve in organic solvent. The heparin-TBA complex formed upon mixing is very soluble in HFIP. Heparin-TBA is demixed from PLCL to form small spherical domains at low concentrations of heparin-TBA, which is similar to co-electrospinning with collagen (Fig. 1-C). The interesting feature of co-electrospun fabrics made of PLCL and collagen or heparin is that these biomacromolecules form spherical dispersed domains in the PLCL matrix phase. Such demixing appears to occur in the evaporation process during the flight of nanofibers in ELSP. This must be due to ionic character of collagen and heparin, both of which were rapidly demixed from nonionic PLCL homogeneous solution during evaporation of volatile solvent to form aggregates, which forms a matrix phase of demixed two-phase blend system. As for the heparin release profile of the nanofiber fabrics, the releasing rate and amount of heparin-TBA released were increased with increasing heparin-TBA content in fibers.

Conclusion: Co-electrospinning of mechano-active biodegradable equimolar PLCL and collagen or heparin, endowing cell binding or anti-thrombogenic potential, facilitates providing a basis for functional scaffold designs for versatile applications in cardiovascular tissue engineering.



Scheme. Schematic of preparation of heparin-TBA salt.

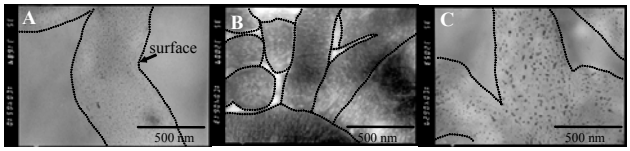


Fig. 1 TEM images of co-electrospun nanofibers made of PLCL blended with (A) 10 wt.%, (B) 50 wt.% of collagen, and (C) 10 wt.% of heparin-TBA