Electrospun Acrylic Terpolymer Nanofibers for Engineered Vascular Replacements

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Introduction: The architecture of an engineered vascular graft plays an important role in promoting tissue ingrowth, reducing anastomotic hyperplasia and favoring the long term patency. Electrospinning produces highly porous microstructures as a network of fine fibers that feature morphological similarity to the extracellular matrix found *in-vivo*. The nanostructure produced by electrospinning process has a high surface area to volume ratio providing for enhanced cell response and potentialy a higher cell density per unit volume compared to other structures. In this work we utilized this process to produce a morphologically relevant vascular graft composed of acrylic terpolymer nanofibers. The cellular responses of human umbilical vein endothelial cells with regard to cell attachment, proliferation, and phenotype expression were investigated.

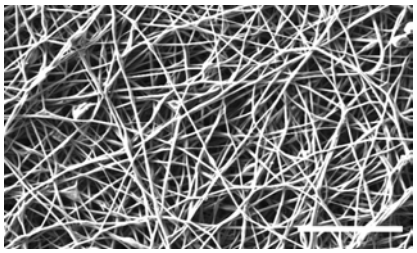
Methods: *Terpolymer Synthesis*: Acrylic terpolymer for this research was prepared by free radical solution polymerization as described in Refs. [1,2].

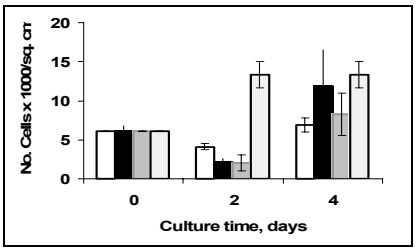
Preparation of Terpolymer Nanofibers: Terpolymer nanofiber mats were produced by an electrospinning method. Briefly, terpolymer solution in acetone (12 wt%) was passed through a needle at a rate 12 ml/hr. The collector was placed at a distance of 20 cm from the needle. A voltage of 30 kV was applied between the needle and the collector. The polymer solution was drawn into fibers by the applied voltage and deposited on the collector in the form of non-woven terpolymer mat. To make oriented terpolymer nanofibers, the fiber was collected on a rotating target.

Cell Culture: Human umbilical vein endothelial cells (HUVEC) were purchased from Cambrex Bio Science (Walkersville, MD). The cells were cultured in endothelial cell basal medium-2, containing growth factors (EGM-2, Cambrex Bio Science). HUVEC passages 4 through 8 were used.

To assess cell attachment and proliferation, HUVEC were seeded on the random in plane and oriented fiber samples, with solution cast terpolymer films and tissue culture polystyrene (TCPS) used as controls. After seeding for pre-determined time periods, cells were stained with DAPI, fluorescence images acquired and cells enumerated using image processing software. The phenotype of endothelial cells was evaluated by assessment of von Willebrand Factor expression by immunofluorescence.

Results / Discussion: Figure 1(top) shows we have successfully spun acrylic terpolymer with a composition HMA:MMA:MAA 90:8:2, from 12 wt% acetone solution. Acetone is a volatile organic solvent; this property promotes solvent evaporation under conventional atmospheric conditions and the deposition of polymer fibers in a nearly dry state. We hypothesize that electrospun terpolymer will produce a surface displaying improved compatibility with endothelial cells. We examined HUVEC growth behavior on electrospun and solution cast terpolymer films and TCPS as a positive control. As seen from Figure 1(middle) electrospun terpolymer surfaces clearly improve cell proliferation compared to a terpolymer film. The results from immunofluorescence staining (Fig. 1 bottom) show that cells growing on electrospun materials highly express vWF.





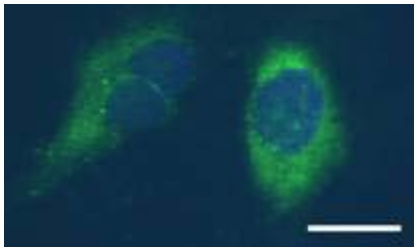


Fig. 1. (top) SEM image of nanofibrous acrylic terpolymer, electrospun from 12 wt% acetone solution. Scale bar-100μm. (middle) Adhesion and growth of HUVEC on electrospun random in plane (black) and oriented (gray) terpolymer nanofibers; solution cast films (white, leftmost bars) and TCPS (dotted, right most bars) were used as controls. (bottom) Expression of von Willerbrand factor by HUVEC seeded on acrylic terpolymer nanofibers. Scale bar- 20 μm

Conclusions: Electrospun acrylic terpolymer surfaces promote favorable endothelial cell responses. Nanofibrous materials improve HUVEC growth and maintain endothelial cell phenotype. This study establishes a baseline prior to the inclusion of phage display selected high affinity peptides into the terpolymer, promoting more precise control over endothelial cell function.

References: [1] Fussell GW, Cooper SL, Biomaterials, 2004; 25:2971-2978. [2] Veleva AN, Khan SA, Cooper SL, JBMR, 2005; 74A; 117-123.