An engineered platform to study the influence of nanotopography on endothelial cell organization

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The endothelium is comprised of cells which line all blood and lymphatic vessels and play a crucial role in maintaining vascular integrity and function. In the body, endothelial cells grow along aligned extracellular matrix (ECM) fibers, typically exhibiting an elongated morphology along the direction of blood flow. Previous *in vitro* studies have shown that the morphology and orientation of endothelial cells are exquisitely sensitive to the composition and topography of the ECM, with nanofibril alignment reorganizing the cytoskeleton along the nanofibril direction and resulting in an endothelial phenotype that is resistant to inflammation. Disruptions in ECM structure are hallmarks of endothelium related diseases, such as atherosclerosis, thrombosis, microvascular diseases, and pulmonary fibrosis. However, there remain gaps in our understanding of the mechanisms by which nanotopographical cues from the ECM modulate cell behavior. To close this gap, we have developed a new class of nanofibrillar supports customized for imaging frozen hydrated cells at the nanoscale with cryo-electron tomography (cryo-ET).

To engineer nanopatterned ECM constructs that are compatible with cryo-ET, we deposited aligned and randomly oriented electrospun ECM fibers on gold transmission electron microscopy (TEM) grids. Electrospinning is a versatile technique for generating fibrous scaffolds that mimic the structure of physiological ECM and affords control over parameters such as scaffold thickness, fiber diameter, and degree of fiber orientation. Here, we electrospun a 28% gelatin solution using a potential difference of 13 kV, a pumping rate of 0.3 mL/hour, a 7 cm distance from the syringe needle, and used a spinning wheel to orient the fibers. We then crosslinked the fibers overnight in 0.05 M EDC to prevent them from dissolving in cell culture media and rinsed them to remove toxic residue. Exposing the TEM grids to atmospheric plasma prior to electrospinning was critical for maintaining adhesion between the fibers and the gold TEM grid surface. We characterized the electrospun fibers on the TEM grids using high resolution scanning electron microscopy (HRSEM), measuring an average fiber diameter of approximately 200 nm (Figure 1). Our immunofluorescence results showed that the shape and orientation of endothelial cells grown on the electrospun TEM grids is influenced by the fiber direction (Figure 2). In addition, we demonstrated that our engineered ECM constructs are compatible with vitrification by plunge freezing and automatic handling within a cryo-TEM. In the future we will use this new class of cryo-ET cell culture supports to investigate the organization of the cell-ECM and cell-cell adhesions that underlie the mechanosensitivity of endothelial cells to changes in ECM topography, ultimately improving our understanding of the mechanical cues required for the formation of healthy tissue and of the pathologies that result from defects in endothelial tissue organization associated with peripheral arterial disease.

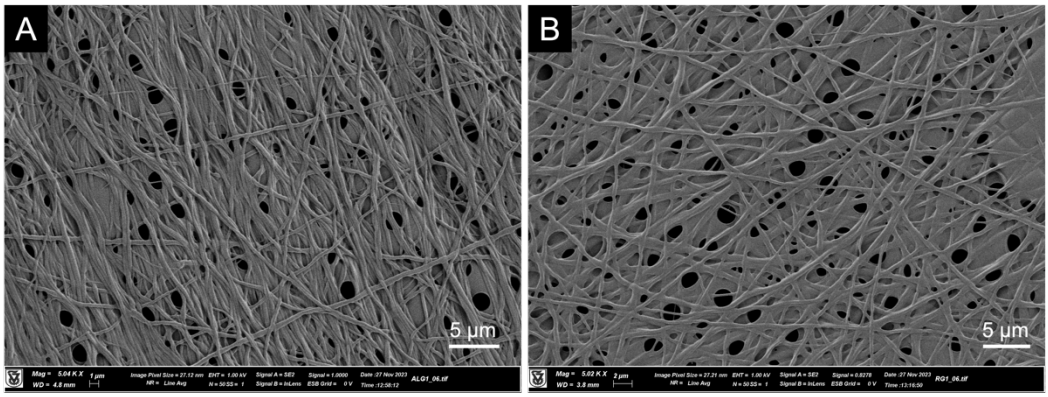

Figure 1: HR-SEM images of gelatin fibers electrospun from a 28% solution directly onto gold TEM grids (UltrAufoil 200 mesh R2,2). (A) Aligned fibers. (B) Randomly oriented fibers.

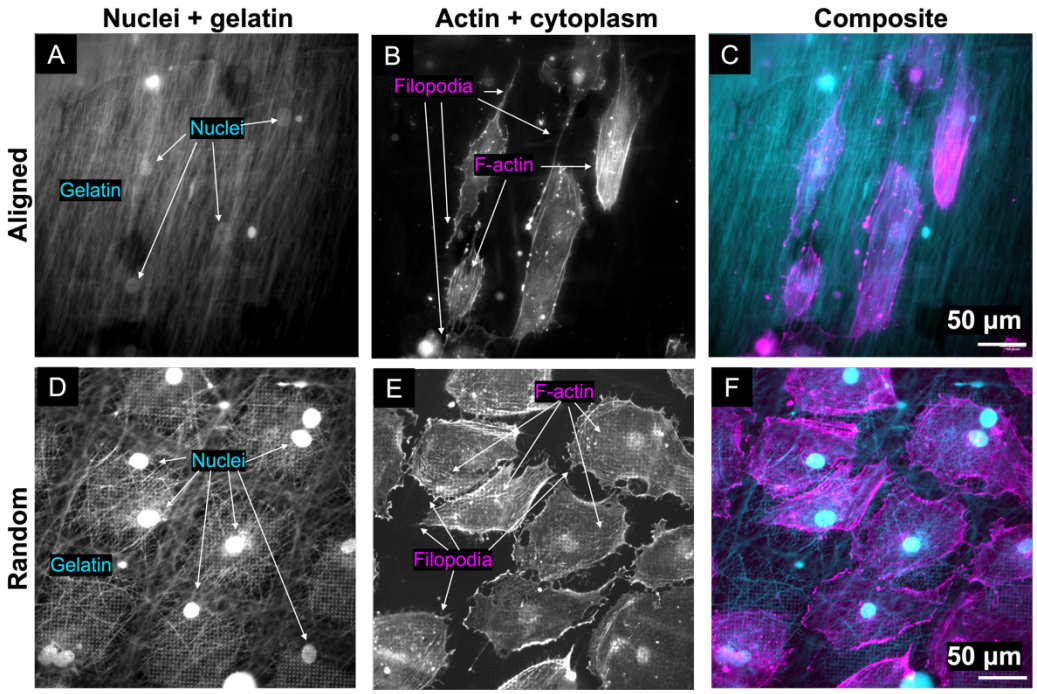

Figure 2: Immunofluorescence of endothelial cells grown on TEM grids coated with aligned (A-C) and randomly oriented (D-F) gelatin fibers for 24 hrs. RFP cells were stained for DNA and F-actin. The aligned (A) and randomly oriented (D) gelatin fibers are autofluorescent and are thus apparent in the 405 nm channel along with the nuclei. (B,D) F-actin along with some background signal from the cytoplasm of the cells. (C,F) Composite images of F-actin (magenta), nuclei and gelatin (cyan) show that the cell morphology and the orientation of F-actin-rich filopodia are clearly influenced by the orientation of the nanofibers.