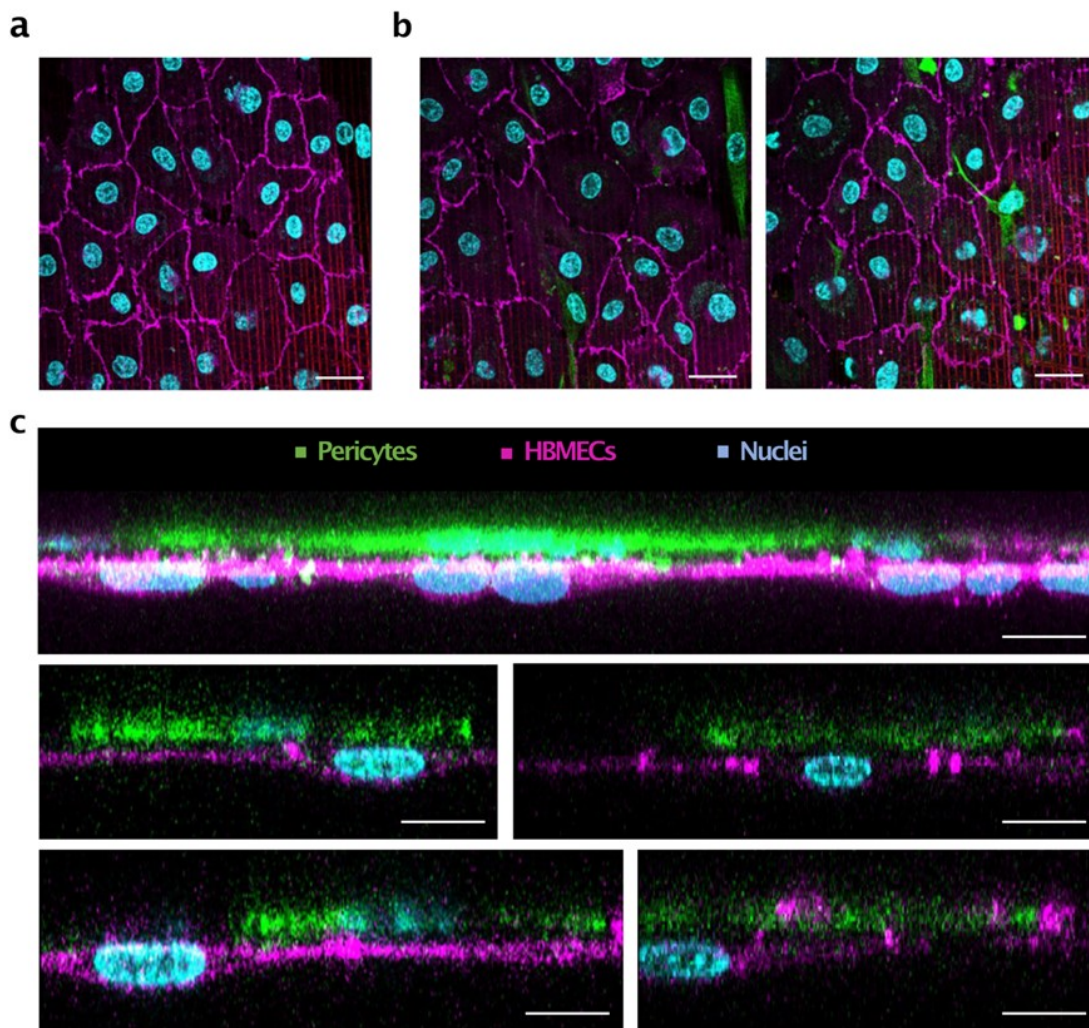
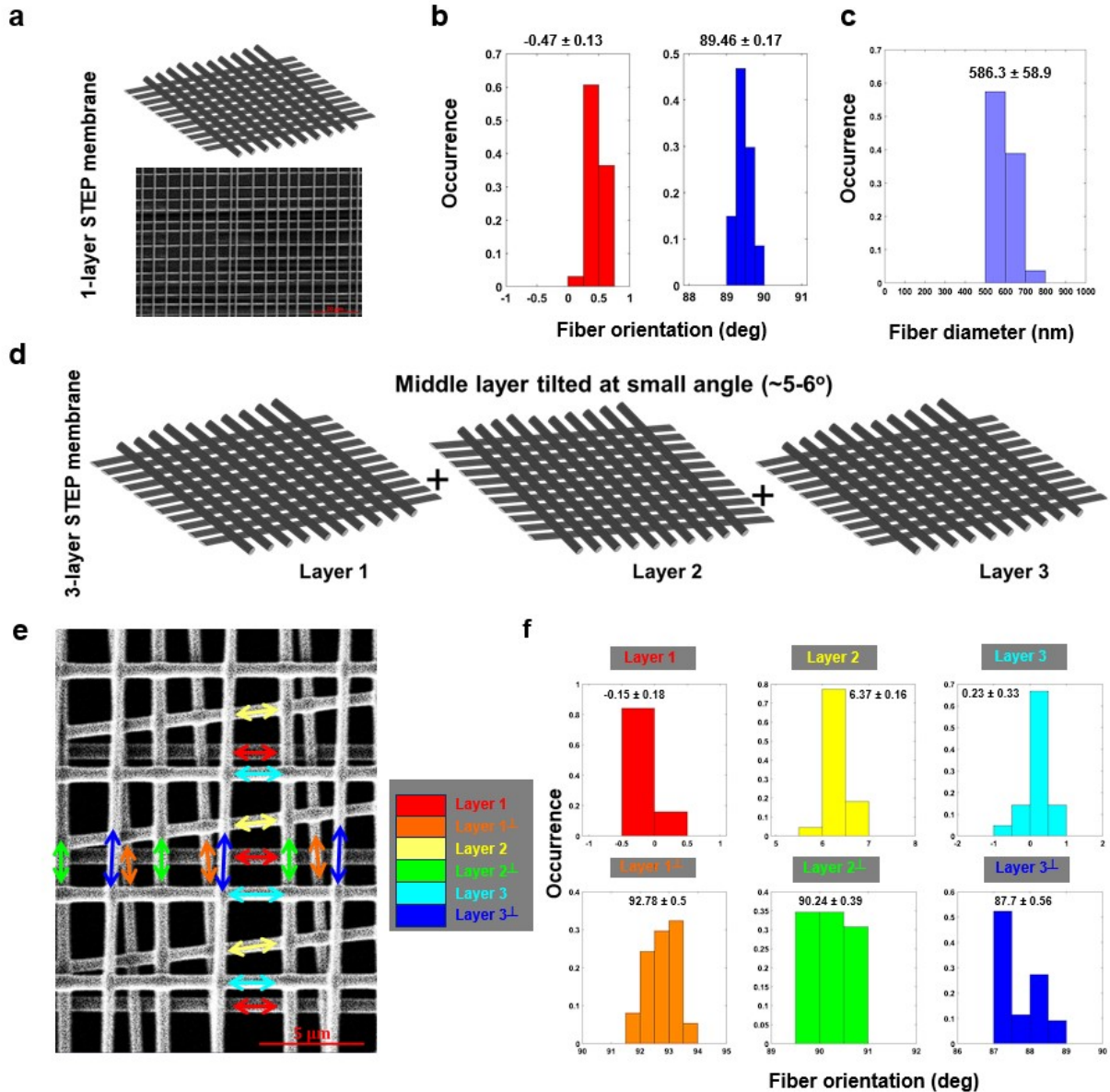


Ultra-Thin and Ultra-Porous Nanofiber Networks as a Basement-Membrane Mimic



Supplemental Figure 1. Additional device images of endothelial cells forming monolayers and co-cultures with pericytes. a) Primary human brain microvascular endothelial cells (HBMECs) cultured in monolayer on nanofibers. Expression of VE-Cadherin (magenta), DAPI (blue), and rhodamine-conjugated fibronectin (red) are shown. b) HBMECs stained with VE-Cadherin and primary pericytes (HBVPs) stained for calponin further demonstrate successful co-culture across nanofiber membranes. Scale Bars 20 μm . c) More Z-stack images of co-cultures in other devices further demonstrate the close-contact of pericytes (green, calponin) and endothelial cells (cyan, VE-cadherin) across the nanofiber membranes. Scale Bars are 10 μm .



Supplemental Figure 2: a) 1-layer STEP membrane, which serves as a base for multi-layer STEP membranes, scale bar represents $20 \mu\text{m}$, b) Histogram analysis of fiber orientation in the 2 fiber directions, values represent average \pm standard deviation, c) Histogram of fiber diameters, d) Schematic of stacking of 3 layers of STEP membranes, e) Zoomed-in image of a 3-layer STEP membrane, colors demonstrate the 3 distinct layers and 2 different fiber orientations, f) Histogram analysis of fiber orientations in different layers, values represent average \pm standard deviation.

Movie 1. Movie of Z-stack images demonstrates the isolation of primary human brain microvascular endothelial cell (HBMEC) monoculture below the nanofiber membrane. VE-cadherin (magenta) demonstrates tight junction formation. DAPI (blue) and rhodamine-conjugated fibronectin (red) are shown.

Movie 2. Movie of Z-stack co-culture images demonstrates the close contact of pericytes (HBVP, green, calponin) and primary human brain microvascular endothelial cells (HBMEC, magenta, VE-cadherin) across the nanofiber membranes. DAPI (blue) and rhodamine-conjugated fibronectin (red) are shown. Many instances of pericyte processes reaching within the membrane are observed without disturbing the endothelial monolayer.

Movie 3. Additional movie of Z-stack co-culture images demonstrates the close contact of pericytes (HBVP, green, calponin) and primary human brain microvascular endothelial cells (HBMEC, magenta, VE-cadherin) across the nanofiber membranes. DAPI (blue) and rhodamine-conjugated fibronectin (red) are shown. Pericyte processes are seen reaching within the membrane.

Movie 4. Movie of Z-stack images across the PET track-etched membrane shows the large separation between pericytes (HBVP, green, calponin) and primary human brain microvascular endothelial cells (HBMEC, magenta, VE-cadherin). DAPI (blue) and rhodamine-conjugated fibronectin (red) are shown. Notice the loss of resolution for the pericytes on the top of the membrane.