Electrospun Nanofiber-supported Collagen Films: in vitro Modeling of Epithelial and Endothelial Tissues

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Statement of Purpose: Impairment of epithelium and endothelium function is responsible for a significant fraction of human pathologies. In vitro tissue culture models that accurately replicate in vivo physiology are essential to further our understanding of disease and to discover and evaluate effective therapies. Several successful in vitro models for such tissues as blood vessel, lung, and glomerulus have utilized commercially-available cell culture inserts with an inert, porous polymer membrane that creates two chambers with separate interacting cell populations. Low porosity and membrane thickness inhibit cell-cell contact between layers that facilitates signaling and gap junction formation. We have developed a thin, highly porous nanofiber-supported collagen membrane that mimics many properties of basement membrane as a replacement for the inert polymer membrane of the cell culture insert.

Methods: Standard procedures were used to generate and characterize electrospun nanofibers. A 10% mass/vol solution of poly-ε-caprolactone (PCL, Mn 70,000-90,000) (#440744, Sigma-Aldrich, St. Louis, MO) in a 55:45 vol/vol mixture of methanol: chloroform was delivered at a flow rate of 0.015 mL/min by a blunt-end needle (Swagelok 316 Ser 1 mm) through a 18 kV field. A sparse, randomly oriented, non-woven mesh of nanofibers was collected on a custom grid made from 24 identical interlocking stainless steel slats. Each 1 mm-thick slat was 210 mm long and 15 mm high with 12 notches spaced 16.25 mm apart (center to center).

Cell culture inserts (#PIXP01250, Millipore, Billerica, MA) from which the provided membranes had been removed were adhered to the fibrous mesh using clear Dow Corning 732 multi-purpose sealant (#732-139ML, Ellsworth Adhesives, Irvine, CA). After the glue had fully cured (24 h), the cell culture inserts were cut away. The inserts were then packaged in 24-well plates and sterilized by ethylene oxide for 3 h at 55 °C.

The mesh on inserts were coated with a sterile solution of collagen I (#3447-020-01, Trevigen, Gaithersburg, MD), diluted 20-fold in 70% ethanol to 0.25 mg/mL. The collagen solution (0.05 mL) was pipetted onto the inner mesh surface of the inserts and air-dried overnight in a laminar flood hood. The inserts were flipped over and coating was repeated on the bottom side of the mesh.

For co-culture models, endothelial cells primary human or rat (HUVEC, primary human sinusoidal, or rat aortic) were first seeded on the outer membrane surface followed by epithelial (primary human hepatocytes, Clara-like NCI-H441 cell line) or smooth muscle cells (primary rat aortic) on the inner surface. The inserts were then placed in the chambers of a 24-well dish with cell type appropriate medium for culture.

Cocultures were assessed by immunoflourescence on intact membranes and histology on sectioned samples. Physiologic assessment (e.g. transepithelial resistance (TER) and vectorial transport) on vital samples was also performed.

Results: SEM was used to evaluate the effects of varying voltage, distance, collection time, and polymer solution on the properties of the nanofibers and resulting meshes. Optimized conditions resulted in meshes with a mean fiber diameter of 287 nm (+/-210) and porosity of 71.3% (+/- 11.7). SEM and thin resin sections demonstrated that the collagen solution dried to form a film with a relatively smooth surface and thickness of 1-2 microns.

All cell types tested readily attached to the collagen film when seeded and grew to confluence. Seeded numbers in co-cultures needed to be determined empirically because of dependence upon relative cell size and proliferation rate. The markers ZO-1 and E-cadherin demonstrated that the cultures quickly formed tight junctions; dexamethasone treatment induced further maturation. These findings were confirmed in TER, vectorial flux and gene expression measurements. Evidence for de novo remodeling of the collagen by the cultured cells was detected.

Conclusions: A synthetic membrane composed of a thin collagen film supported by a sparse mesh of electrospun nanofibers was created to better mimic the properties of the basement membrane for in vitro tissue culture models. It provides support and barrier function to developing epithelium and endothelium without inhibiting free diffusion of small molecules. The resulting cellular constructs were sufficiently durable to be able to be probed with antibodies and stains for microscopy. The membrane could enable improved physiological modeling of tissues for drug discovery or scientific investigation.