Polycaprolactone Electrospun Fibers to Modulate Basement Membrane Remodeling in Upper Airway Coculture

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Statement of Purpose: The functionality of the upper airway mucosal layer is highly affected by the basement membrane which is the spatially dense extracellular matrix under the epithelium lining[1, 2]. Understanding the impact of culture substrates on basement membrane regulation in in vitro models and the impact of mechanical and morphological cues on basement membrane function is crucial for physiological microenvironment models. In this research, we investigate the underlying physiology and model the microenvironment of the epithelial-mucosal lining of the trachea by establishing an in vitro microphysiological model using Polycaprolactone (PCL) electrospun fibers. In this model, PCL fibers were used as a substrate for epithelial-fibroblast co-culture in order to mimic the mucosal layer and the basement membrane of the trachea and recapitulate the biophysical milieu of the native tissue.

Methods: Polycaprolactone (PCL) fibers were electrospun from PCL pellets dissolved in chloroform and ethanol (98:2 v/v) in either an aligned or randomly oriented configuration. The membranes were then assembled in a transwell-like (Figure 1) configuration and cultured with human tracheal fibroblast for 7 days followed by human bronchial epithelial cells to generate a coculture (CC). To induce epithelial differentiation, an air-liquid interface was introduced at Day 14 and culture continued for 28 days. Fiber morphology, local mechanical properties of the matrix and cell-coculture, immunohistochemistry, histological analysis, and RNA sequencing were conducted to understand matrix and tissue evolution within the micro-environment.

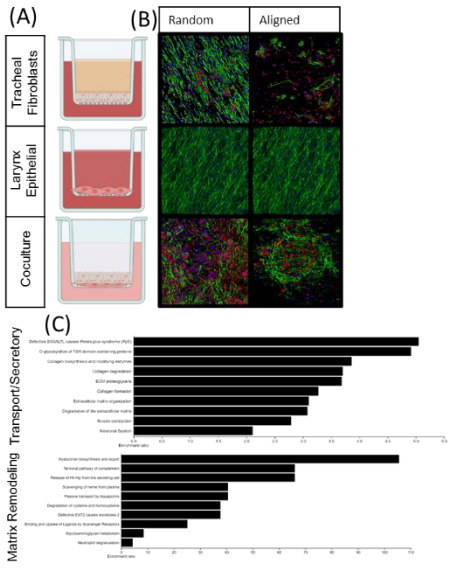

Figure 1: (A) Schematic picture of the coculture at liquid-air interface. (B) IHC images of fibroblasts, epithelial, and coculture at day 21. (C) RNASeq demonstration.

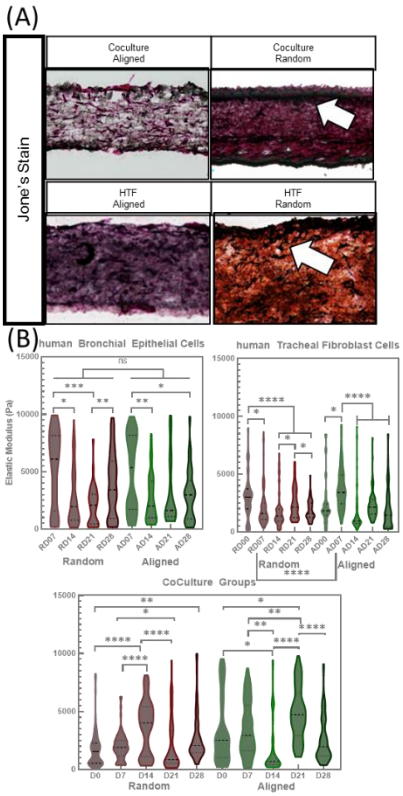

Figure 2. (A) Histological Observation. Basement membrane staining conducted using Jones' staining on Random (RCC) and Aligned (ACC) coculture at day 21. (D) Local elastic modulus of cocultured groups at day 0, 7, 14, 21, and 28 in RCC and ACC.

Results: The development of the epithelial mucosa and corresponding changes in mechanical stiffness in both membranes could be identified by in situ indentation (Fig 1). Coculture systems upregulate matrix remolding genes (mmp2, wnt and sox2) while it downregulates fibrotic scarring in bmp and col1 signaling. In addition, the system matures in 14 days and the exposure to the air-liquid interface is immediately noticed in the TEER measurements. Mature basement membrane with functional mucosal membrane was produced using bilayer membrane; however, there is no significant difference observed in RCC group compared to ACC. The Random fibers showed trends of not encouraging a fibrotic phenotype, while the aligned fibers showed significantly greater basement membrane stiffness as well as early increase in trans-epithelial resistance.

Conclusions: A coculture system was developed to model the tracheal mucosal microenvironment using fibers that mimicked the basement membrane, leading to functional 3D in vitro model that mimicked tissue mechanics and transport function as well as cell-cell communication.

References: 1. Tam, Anthony, et al. Therapeutic advances in respiratory disease 5.4 (2011): 255-273.