Design and Evaluation of *In Vitro* Lung Tissue Models for Antigen Delivery

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Statement of Purpose: The respiratory mucosal interfaces serve as the primary line of defense for antigen entry, from the environment to the local tissue leading to systemic circulation. The physiological response of the mucosal barrier is of direct relevance to drug delivery and understanding disease pathogenesis. Specifically, the epithelial tissue tight junctions serve as entry gates to paracellular and intracellular pathways. The local peripheral vasculature also serves as a barrier at these interfaces. The mucosal-vasculature interface serves as a protective measure to regulate foreign body recognition and entry. The efficiency of this interaction is relevant to therapeutic delivery and disease pathogenesis (Tonnis, 2013; Kreijtz, 2011). Reproducing mucosal entry pathways is critical to developing biologically relevant *in vitro* biomimetic tissue models composed of primary human cells and cell lines. To mimic local respiratory physiology, our *in vitro* primary cell models of upper (nasal) and lower (alveolar) airway consist of mucosal epithelium and endothelium. Our stepwise approach to evaluate tissue barrier function begins with studying baseline transepithelial electrical resistance (TEER), along with barrier responsiveness to monocyte addition and known respiratory immunopotentiators. The tissue barrier permeability was characterized by controlled molecular mass transfer studies, across five human mucosal tissue equivalent (MTE) model systems.

Methods: Tissue models consisting of primary human nasal and alveolar cells (PromoCell), A549 (ATCC) and CRL-1848 (ATCC) were prepared with cell type specific media and extracellular matrix proteins (collagen, fibronectin, and laminin). The experiments were staggered to take into account the tissue confluence rates (3-days for cell lines; 5-7days for primary cells). The barrier function studies were done after tissue differentiation confirmation via high resolution microscopy. STX2 Endohm6 (World Precision Instruments.) voltammeter was used to for transepithelial electrical resistance (TEER) readouts of MTE constructs. The normalized TEER values were determined by subtracting the blank transwell readout from the MTE containing chambers, in the respective media. To verify MTE stability, ten MTE were tested alongside ten controls to get a baseline MTE TEER reading. The effect of immunopotentiators on the tissue electrical resistance was tested with the addition of peripheral blood mononuclear cells (PBMC) to the MTE modules. Each condition was tested with 12 replicates in multiple plate set-ups to characterize well-to-well variation. The tissue constructs were washed with base media prior to the addition of one million PBMC (90 min, 37^{o}C, 5%CO_2). TNFα (10ng/ml) or Dexamethasone (10ng/ml) was added to the apical side and TEER readouts were taken after 2hr incubation. For the mass transfer studies, FITC-Dextran permeability was assessed as a secondary measure to quantify tissue integrity. Serial dilutions of FITC-Dextran (10KDa, 70KDa, 500KDa; Sigma) were added to the apical side of MTE. Apical and basal media from eight MTE modules were tested at 485/535nm wavelength. Dextran permeability was quantified over 30, 180, and 360 minute intervals.

Results:
Based on the barrier function related electrical resistance and molecular permeability quantitative measures, we found that the primary human alveolar bilayer model is a stable tissue motif with sensitivity to both pro-inflammatory (TNFα) and immunosuppressive (Dexamethasone) mediators. The bilayer model also has the least level of short-term Dextran permeability; this illustrates the presence of active tissue tight junctions. The primary alveolar epithelium alone showed the most molecular weight dependent permeation of FITC-Dextran (10KDa>70KDa>500KDa). Of the primary cell models, the nasal epithelium model showed the least degree of Dextran size based permeation. This demonstrates the intrinsically leaky nature of the differentiated nasal mucosa. In comparison, the cell line models showed a lack of tissue electrical resistance stability and the highest degree of molecular weight independent, non-selective FITC-Dextran permeation. There was also more well-to-well and plate-to-plate variation in the cell line models. The cell line MTE response to immunopotentiators was not comparable to the primary cell MTE systems. The results demonstrate the barrier function differences in local respiratory physiology of upper and lower respiratory mucosal sites, the knowledge of which is important for developing physiologically relevant biomimetic model systems.

Conclusions: The evaluation of intrinsic differences and similarities between primary human nasal and alveolar cells and cell lines holds the potential for validating high throughput drug immunoscreening *in vitro* tissue models. These respiratory tissue barrier function studies demonstrate differences between cell lines and primary cells in terms of baseline electrical resistance, immunopotentiator responsiveness, and FITC-Dextran permeability. These preliminary findings will be used for future work with liquid and aerosolized antigen delivery in our *in vitro* respiratory tissue model systems.

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References:
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