Hierarchical Nanostructured Polymer Films Enhance Protein and Antibody Permeability

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Statement of Purpose: The tight junction barrier of epithelial and endothelial tissue presents a major obstacle in targeted drug delivery for systemic circulation. A number of tight junction modulators have been investigated over the years to increase permeability for drug delivery applications. These strategies have included chelators, fatty acids, copper, iron, and peptides among others to facilitate tight junction opening between cells (1, 2). Here, we present a novel approach to increase permeability and enhance paracellular transport across a confluent Caco-2 cell monolayer via a purely topographical cue rather than through biochemical strategies.

Methods: Nanostructured films with features exhibiting fractal geometry on the nano to micro length scale were fabricated through a nano-templating technique using polystyrene and polypropylene. A total of 6 different fractal geometries were fabricated. The presence of the features was verified through scanning electron microscopy (SEM).

Permeability studies were performed on the human intestinal Caco-2 cell line. Cells were seeded on translucent inserts with a high density of pores and grown to confluency until tight junctions formed as measured by the trans-epithelial electrical resistance (TEER) technique. Nanostructured films were placed in contact with the cell monolayer and a solution of bovine serum albumin conjugated to FITC (66 kDa) or immunoglobin G conjugated to FITC (150 kDa) (BSA-FITC, IgG-FITC) was added to the apical side of the well inserts. The solution on the basal side of the insert was sampled over 2 hours and contained the BSA-FITC or IgG-FITC that was transported across the Caco-2 monolayer. The concentration of BSA-FITC and IgG-FITC was measured against a standard curve using a fluorometer.

Confocal microscopy was employed to image the distribution of BSA-FITC and IgG-FITC in relation to the Caco-2 cells to visualize the permeability pathway. Additionally, since F-actin depolymerization disrupts tight junction structure and barrier function, phalloidin-FITC was used to stain for F-actin. Cells were first fixed in paraformaldehyde, permeabilized with Triton X, and then stained with phalloidin-FITC.

Protein adsorption studies were performed to characterize the surface energy of the nanostructured films. The assay involved labeling fluorescent dye to the proteins of interest, incubating the labeled proteins with the film, and then washing away the unadsorbed protein using PBS. The amount of adsorbed protein was measured using fluorescent microscopy.

Results: The nanostructured films were successfully fabricated with batch-to-batch repeatability as verified by SEM. The concentration of BSA-FITC and IgG-FITC on the basal side of the well inserts significantly increased at 2 hours for the nanostructured DN2 film compared to the unimprinted flat film and to the cells alone as seen in Fig. 1. Additionally, confocal microscopy suggests that the BSA and IgG is transported through the cell monolayer via both the transcellular and paracellular pathways as seen in Fig. 2. The effect of the nanostructured films on F-actin will be investigated to elucidate a mechanism for the observed enhanced permeability.

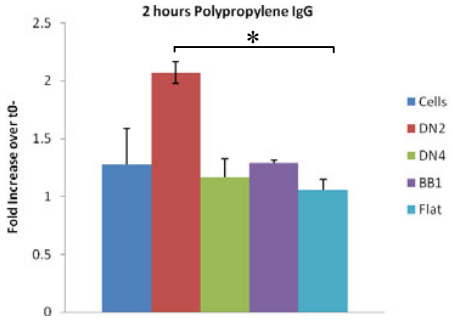

Figure 1. The DN2 polypropylene structure exhibited increased concentrations of IgG-FITC after 2 hours.

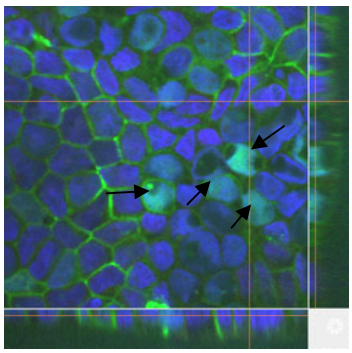

Figure 2. The structures induce both transcellular (arrows) and paracellular transport across the Caco-2 monolayer. (Green = IgG-FITC, Blue = Caco-2 cells)

Conclusions: Here, we demonstrated that the permeability across a monolayer of confluent Caco-2 cells is increased more than two-fold over the flat unimprinted control film. To the best of our knowledge, we show for the first time that a nano-topological cue has been used as a permeability enhancer. Future work includes investigating the mechanism for this phenomenon through mechanotransduction studies. This approach has an advantage over biochemical cues due to the low-cost and repeatability of nano-templating. Combined with a drug payload, this nanostructured surface could be exploited for future drug delivery vehicles.

References:

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2. Deli MA. Potential use of tight junction modulators to reversibly open membranous barriers and improve drug delivery. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2009 4;1788(4):892-910.