Epithelial Cell Response to a Chemically Modified, Flexible Substrate, Presenting Nanoscale Topographic Features

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Statement of Purpose: The basement membrane *in vivo* presents a flexible, felt-like nanoscale topography and complex chemical cues to attached cells. Previous work has shown that chemically uniform anisotropic topography, consisting of ridges as small as 70 nm with a spacing of 400 nm etched into silicon, induces many changes in cell responses compared to flat or micron-scale topography, including orientation, mobility, adhesion, differentiation, and g-protein signaling. However, exploration of appropriate random and/or flexible controls has been somewhat limited due to a lack of availability of chemically uniform or flat controls. By using track-etched polycarbonate membranes as a template for electroless deposition, a random, biologically compatible nanoscale topography consisting of flexible gold nanotubules projecting from a polycarbonate surface can be used to explore the combined effects of topography, chemistry, and flexibility on the behavior of human corneal epithelial cells (HCECs)

Methods: Gold nanotubules were fabricated by dipping a carbon-sputtered polycarbonate membrane in a series of metallic salt baths, which leaves an all-over coating of gold approximately 50 nm thick. After removing the topmost layer of gold, etching the polycarbonate membrane with oxygen plasma, a series of gold nanotubules can be seen projecting from the polycarbonate surface with an outer diameter that depends on the diameter of the pores in the membrane.

The gold surface was modified using mercaptohexadecanoic acid, whose thiol group reacts with the gold to form an acid-terminated self-assembled monolayer, which encourages cell adhesion and spreading.

Sparsely plated cells were analyzed to determine cell viability, morphology, adhesion, and proliferation.

Results / Discussion: Substrates suitable for cell culture, with nanotubules ranging between 50 and 800 nm in outer diameter, 10 to 700 nm in inner diameter, and with an average spacing of between about 440 and 2000 nm, respectively, were fabricated in a high-throughput manner, yielding large (10cm x 10cm) regions of uniform topography.

Primary HCECs plated on nanotubule surfaces (220nm outer diameter) were present in comparable numbers with flat electroless and evaporated gold surfaces after 24 hours, indicating that the cleaning steps undergone were sufficient to allow cell viability. Cell area was decreased on nanotubule surfaces compared to flat; however, examination of filopodia by SEM revealed that cells on nanotubule substrates extend a great number of filopodia which attach directly to the gold nanotubules, bending them as the cell moves across the surface. This morphology is comparable to epithelial cells attached to the basement membrane *in vivo*.

Conclusions: Gold nanotubule substrates provide a easy-to-fabricate substrate with flexible nanoscale topography that encourages extension of cellular processes and cellular growth. The ability to vary the size and thickness of nanotubules by choice of membrane and plating conditions allows for the fine-tuning studies on the combinatorial effect of substrate flexibility and nanoscale topography. The ability to readily chemically modify the gold using self-assembled monolayers of alkanethiols, combined with the large areas that can be patterned, allows for studies of mechanisms induced via initial binding events.

References:

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