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EFFECTS OF MICRO-AND NANOSCALE SUBSTRATE TOPOGRAPHIES ON THE BEHAVIOR OF HUMAN CORNEAL EPITHELIAL CELLS

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INTRODUCTION: Epithelial cells adhere to specialized extracellular matrices called basement membranes, composed mainly of type IV collagen, laminin and heparan sulfate proteoglycan, that provide physical support and present chemical cues to cells. The human corneal epithelial basement membrane was found to have a felt-like appearance with pores and fibers with lateral dimensions ranging from 22nm to 191nm[1]. We hypothesize that substrate topography of nanoscale dimensions, independently of chemistry, affects the behavior of human corneal epithelial cells. Many cell types align along anisotropic topographic features such as patterns of grooves and ridges, a phenomenon called contact guidance[2]. We analyzed the morphology and orientation of cells cultured on patterns of grooves and ridges with nanoscale dimensions and compared them with the cellular responses to micrometer sized grooves and ridges and to smooth substrates.

METHODS: A layer of UV3 photoresist (Shipley) was coated onto silicon wafers and patterned using electron-beam lithography. The resist patterns were transferred to the underlying silicon by reactive ion etching. The resist remaining after etching was removed and the wafers were coated with a layer of silicon oxide in a Low Pressure Chemical Vapor Deposition reactor. Each substrate consisted of an array of six $4mm^2$ patterned fields, separated by smooth areas. The pattern dimensions within each field were uniform. The pitch of the features in the different fields ranged from 400nm to 4000nm in each substrate (pitches equal to 400nm, 800nm, 1200nm, 1600nm, 2000nm and 4000nm).

Human corneal epithelial cells were harvested from corneas donated by the Lions Eye Bank of Wisconsin. After reaching 80% confluence, the cells were suspended in SHEM medium supplemented or not with 10%(v/v) of Fetal Bovine Serum. Cells were plated at a density of 8,500 $cells/cm^2$ in 24 well plates containing the patterned silicon substrates, previously sterilized with 70% ethanol.

After a 12-hour incubation F-actin and the nuclei were stained with TRITC-phalloidin and DAPI, respectively. Images of the stained cells were obtained from an epifluorescence microscope and were analyzed using Metamorph™ software (Universal Imaging Corporation).

Cells prepared for electron microscopy observation were fixed in glutaraldehyde, post-fixed in osmium tetroxide, dehydrated in graded ethanols, immersed in hexamethyldsilazane and coated with platinum.

RESULTS: A sub-population of the human corneal epithelial cells cultured on substrates patterned with 70nm wide ridges on a 400nm pitch aligned along the direction of the topographic features. Aligned cells were elongated compared with cells cultured on the smooth substrates, which were mostly round. Cells on the patterned substrates that were not aligned were round and were frequently poorly spread. Cell elongation and alignment occurred on all patterns tested. The percentage of aligned cells was constant for pattern pitches ranging from 400nm to 2000nm, and was lower for 4000nm pitch features, on 600nm deep grooves. When the groove depth was decreased to 150nm, the percentage of aligned cells was constant for all pitches tested and was lower than on 600nm deep grooves for pitches from 400nm to 2000nm. Filopodia were able to adhere to both grooves and ridges. The topographic features frequently guided filopodial orientation. Lamellipodia bridged the grooves except at the cell edge along the patterns where lamellipodia often protruded into the grooves.

DISCUSSION & CONCLUSIONS: We have found that human corneal epithelial cells react to topographic features with dimensions similar to those found in the native basement membrane. Our findings may be important in the design of systems for cell culture, tissue engineering and the development of implantable prosthetics.

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