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Structural Organization of the Cytoskeleton in SV40 Human Corneal Epithelial Cells Cultured on Nano- and Microscale Topography

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The basement membrane of human corneal epithelial cells possesses a three-dimensional nanoscale topography composed of pores, bumps and fibers with typical dimensions of 50 to 240 nm [1]. A number of studies have demonstrated the capacity of substrate topography to influence a number of cell functions in human corneal epithelial cells such as cell morphology, cell-substrate adhesion and proliferation [2-4]. This report demonstrates that substrate topography influences cytoskeleton organization and the distribution of $-\beta_1$ integrins in SV40 human corneal epithelial cells (SV40-HCECs).

Silicon chips containing anisotropic topographies in the form of uniform groove and ridge patterns and isotropic topographies in the form of cubic arrays of holes were created using lithographic techniques. Each type of topography, had features ranging from the biomimetic length scale, the 400 nm pitch, to the microscale, the 4 μm pitch. SV40-HCECs were cultured on the silicon chips for a 24-hour period and the organization of cytoskeletal elements and the distribution of immunogold labeled $-\beta_1$ integrins was imaged using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Cells cultured on the groove and ridge patterns were aligned and elongated in the direction of the underlying patterns (Fig. 1A). Cytoskeletal elements aligned with the direction of the grooves (Fig. 1B & 1C) and the spatial organization of the cytoskeleton was influenced by groove size. In cells cultured on the groove-ridge patterns, $-\beta_1$ integrins were concentrated at the poles of the spindle shaped aligned and elongated cells (Fig. 1D). Compared to cells on the microscale hole patterns and on the planar surface which were more evenly spread, individual SV40-HCECs on the 400 nm pitch hole patterns possessed a stellate morphology (Fig. 2A). Qualitative analyses of transmission electron micrographs of the basal surface of SV40-HCECs immunogold labeled for $-\beta_1$ integrins demonstrated that cells on nanoscale holes exhibited longer and more numerous filopodia structures than cells cultured on microscale holes and these structures were associated with $-\beta_1$ integrins (Fig. 2B). Cross-section studies demonstrated that cell contact with the substrate in both groove and ridge patterns and hole was restricted to the tops of the ridges.

Physical features introduced by substrate topography present overlying cells with adhesive and non-adhesive regions for cell spreading which is reflected in the spatial organization of the cytoskeleton on the different groove and ridge features. The cytoskeleton plays pivotal roles in cell shape, cell-substrate adhesion and cell proliferation. Cells cultured on biomimetic nanoscale grooves exhibit increased cell-substrate adhesion compared to cells cultured on microscale grooves and planar

substrates [2]. Subsequently, the tuning of certain mechanical aspects of materials such that they mimic the natural environment of cells opens the possibility for the successful design of more in vivo like cell culture systems and for improved biomaterials.

References

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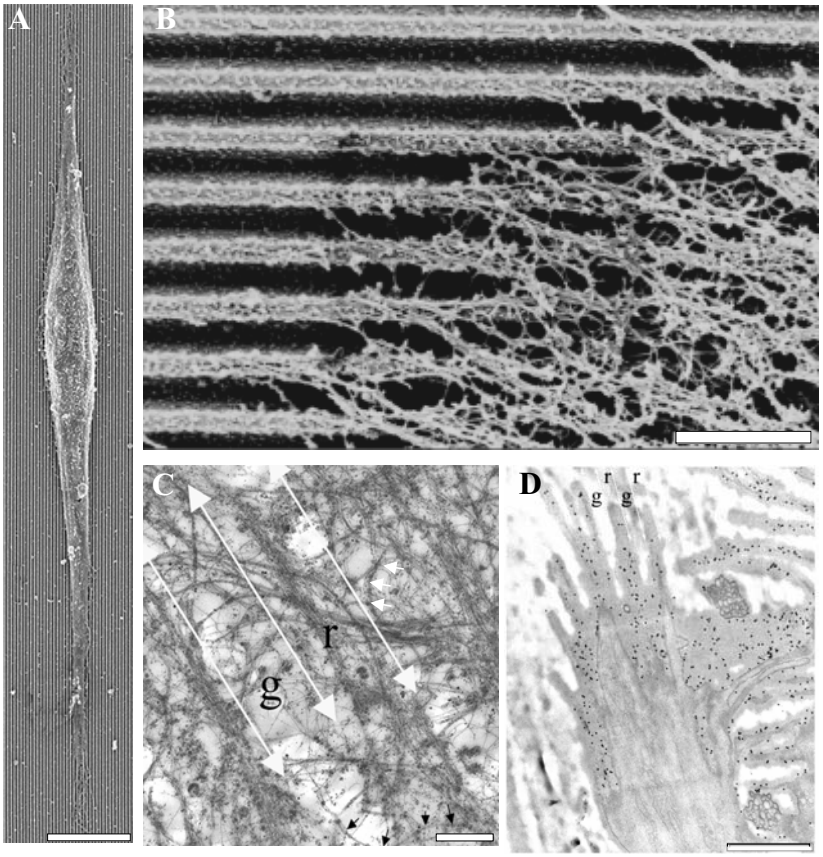

Fig. 1: Response of SV40-HCECs to groove-ridge topography imaged by scanning and transmission electron microscopy. (A) & (B) respectively show contact guidance and the organization of peripheral cytoskeletal elements on the 400 nm pitch; (C) Organization of basal cytoskeletal elements in the 1200 nm pitch; (D) Distribution of $-\beta_1$ integrins at the basal surface of a cell on the 400 nm pitch. Letters 'g' and 'r' in (C) and (D) represent groove and ridge positions. Double headed arrows show direction of

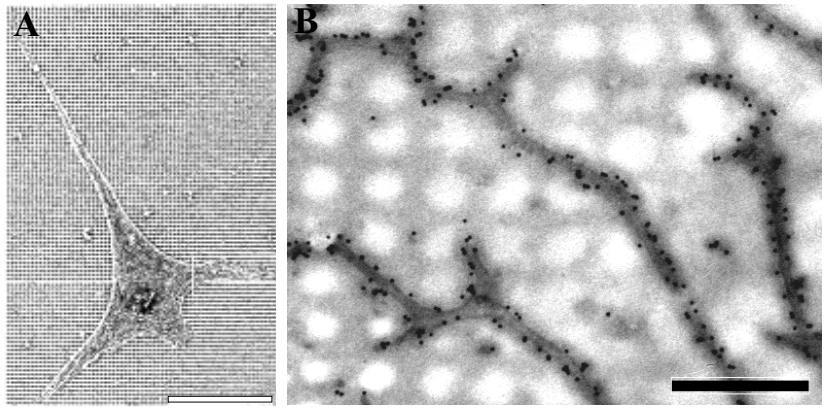

Fig. 2. Response of SV40-HCECs to cubic arrays of nanoscale hole patterns imaged by scanning and transmission electron microscopy: (A) Cells on the 400 nm pitch exhibit stellate morphology; (B) Basal surface of SV40-HCECs has numerous filopodia extending over the surface with $-\beta_1$ integrins. Scale bar in A and B

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