Immobilized Integrin and Syndecan Binding Peptides Co-Modulate Human Corneal Epithelial Cell Response To Nanoscale Topography

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Statement of Purpose: An effective approach to biomaterials takes advantage of the synergistic effects of nanoscale topography, chemistry, and compliance. We have previously demonstrated the effects of nanoscale features on a variety of cellular behaviors. In order more fully understand cell-level control *in vivo*, we employ a systems approach which looks at the effect of nanoscale topography in conjunction with surface chemistry.

It has been hypothesized that cellular response to nanoscale topography is mediated by the initial cellular attachment to a surface. Previous work in our laboratory has shown that human corneal epithelial cells (HCECs) response to nanoscale topography is affected by changes in cell media. This response may be due to differences in the adsorbed protein layer, resulting in different cell-adhesive groups being made available to attached cells.

Our approach uses flat or topographically patterned substrates coated with gold and modified with an oligo (ethylene glycol) self-assembled monolayer (SAM), a small percentage of which can be linked to a peptide that attaches to integrin or syndecan. The oligo(ethylene glycol) background prevents nonspecific protein adsorption, thus allowing the cells to only interact with the peptide groups of defined composition and spatial concentration.

Methods: Peptide modified surfaces were prepared as described in Clare and Abbott. Briefly, gold-coated surfaces (Platypus Technologies, Madison, WI) were exposed to 0.2 mM oligo (ethylene glycol) (Prochimia, Gdansk, Poland) containing a defined percentage of amine termination. The amine-terminated groups reacted with a 2 mM solution of sulfo-SMCC (Pierce Biotechnology, Rockford, IL), then were placed into 100 µM peptide (CGGRGDS, CRKRLQVQLSIRT, or scrambled sequences) solution. Unreacted linker groups were quenched in 2 mM 2-mercaptoethanol. Protein adsorption to these materials was tested by placing SAM-modified surfaces into fetal bovine serum for 2 hours, and by measuring the change in thickness with ellipsometry. Surfaces used in cell culture experiments were sterilized under a UV-A lamp. Primary HCECs were harvested and plated in Epilife™ (Cascade Biologics, Portland OR) at a density of 10,000 cells/mL. After 3, 6, or 24 hours, cells were fixed in 4% paraformaldehyde, stained with DAPI and TRITC-phalloidin, and imaged by fluorescence microscopy. A centrifugal assay was employed to determine cell's adhesive strength.

Results/Discussion: *Protein adsorption*: At initial amine percentages less than 1% (average peptide spacing=4.8 nm, assuming complete reaction), the thickness of the adsorbed protein layer was measured to be less than 1nm, which was insufficient to sustain cell attachment alone. Negative controls, consisting of scrambled peptides at coverages less than 1%, pure oligo (ethylene glycol), and surfaces terminated with only 2-mercaptoethanol, did not support cell attachment. Therefore, we focused on amine percentages less than 1% to ensure that cell response was entirely due to the defined peptide surface.

Cell attachment and spreading: Cell adhesion to both integrin- and syndecan-binding peptide modified SAMs occurred at any peptide coverage greater than 0.01%, comparable to work by Roberts et al. Interestingly, there was little variation in cell number, size, shape, or adhesion between 0.01 and 1% for either integrin or syndecan-binding peptide surfaces, indicating a comparable number of these binding moieties on the cell surface. There was a 20% increase in cell attachment on surfaces modified with syndecan-binding peptides.

Nanoscale cell response: Preliminary work with micron- and nano-scale topographic features modified with integrin-, syndecan-, or integrin- and syndecan-binding peptides have shown that each promote unique cellular responses to nanoscale topography. The presence of both peptides at the surface are required to facilitate cell spreading and alignment comparable to that on non-defined surfaces. Co-presentation of a syndecan ligand modulates the integrin-mediated alignment responses.

Conclusions: Well-defined surface chemistries, consisting of SAMs of peptide-terminated oligo (ethylene glycol), were fabricated by facile and flexible synthethic methods, which have the advantage of being able to test many peptides without undergoing a separate synthesis for each condition. The SAMs fabricated by this strategy are comparable to those made by other methods, and can be used in studies in which a well-defined surface chemistry is required.

References:

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