Arrays of Topographically and Peptide-Functionalized Hydrogels for Analysis of Biomimetic Extracellular Matrix Properties

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Epithelial cells reside on specialized extracellular matrices that provide instructive cues that regulate and support proper cell function. We have previously demonstrated that substrate topography with dimensions similar to the native extracellular matrix (submicrometer and nanoscale features) significantly impacts corneal epithelial proliferation[1] and migration[2]. Here, we investigate the incorporation of additional instructive cues to include specific peptide ligands on topographically nano-patterned hydrogels. The efficient, systematic study of multiple instructive cues (peptide, peptide concentration, topographic dimensions) is contingent on the development of high throughput platforms.

On the materials side, we have developed synthetic techniques to realize hydrogel materials that mimic the biochemistry of the native extracellular matrix, including the incorporation of multiple relevant peptide epitopes. Specifically, distinct functional pegylated peptide ligands were synthesized (Figure 1) and incorporated into an inert hydrogel network[3]. Using these materials, medium throughput arrays were developed for cell culture that enabled the investigation of a large parameter space while also reducing the amount of material consumption. Array templates were generated from lithography molded elastomeric stamps and used to constrain the spatial location of each experimental condition (Figure 2 and 3). These medium throughput arrays were used to systematically and rapidly evaluate combinations of two different peptide motifs (RGD, AG73) on corneal epithelial behavior, including cell proliferation, cell spreading and initial cell attachment.

We have incorporated an additional instructive element by molding nanometer sized topography on the surface of these peptide functionalized hydrogels. Large area elastomeric substrates generated by soft lithography techniques were used to pattern large areas of hydrogel arrays (Figure 4). The topographically and peptide-functionalized hydrogels are used to characterize single cell migration and the proliferation and wound healing response of confluent cells.

1. S. Liliensiek, S. Campbell, P. Nealey and C. Murphy, J Biomed Mater Res A **79** (2006).
2. K. Diehl, J. Foley, P. Nealey and C. Murphy, J Biomed Mater Res A **75** (2005).
3. M. Wilson, S. Liliensiek, C. Murphy, W. Murphy and P. Nealey, Soft Matter **8**, 2 (2012).

1)

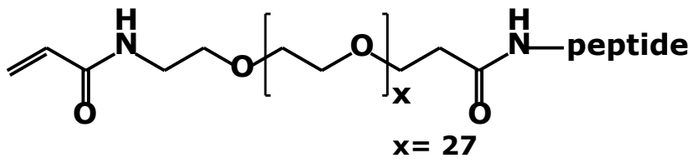

Figure 1. Chemical structure of the functional pegylated peptides synthesized via solid phase synthesis and purified with HPLC (purity>80%).

2)

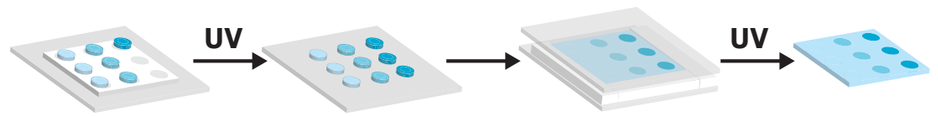

Figure 2. Schematic of the medium throughput arrays that were generated from elastomeric templates. Functionalized peptide conditions were spotted in the array and exposed to UV prior to the removal of the template. The array was then backfilled with an inert hydrogel material and exposed to UV.

3)

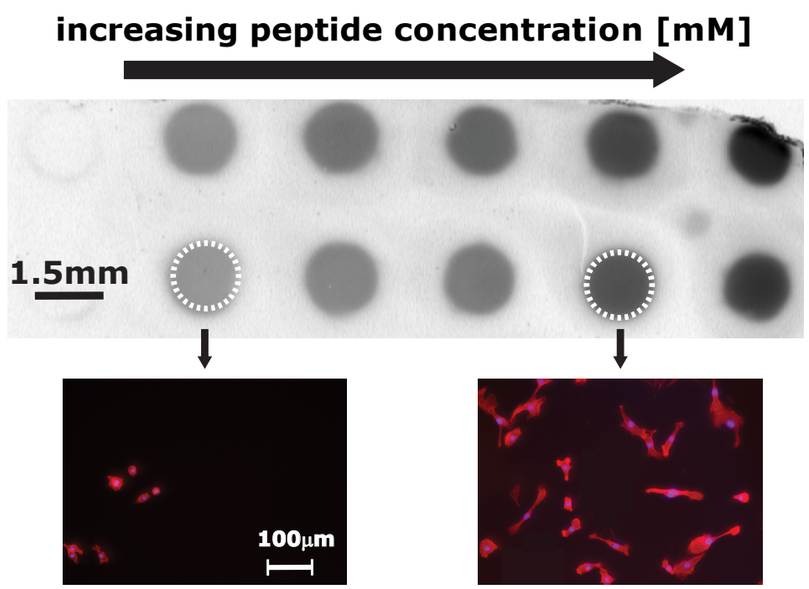

Figure 3. Fluorescent scanner image of the hydrogel arrays functionalized with different concentrations of fluorescently tagged pegylated peptides. Representative fluorescent images of corneal epithelial cells that were seeded on hydrogels at different peptide concentrations.

4)

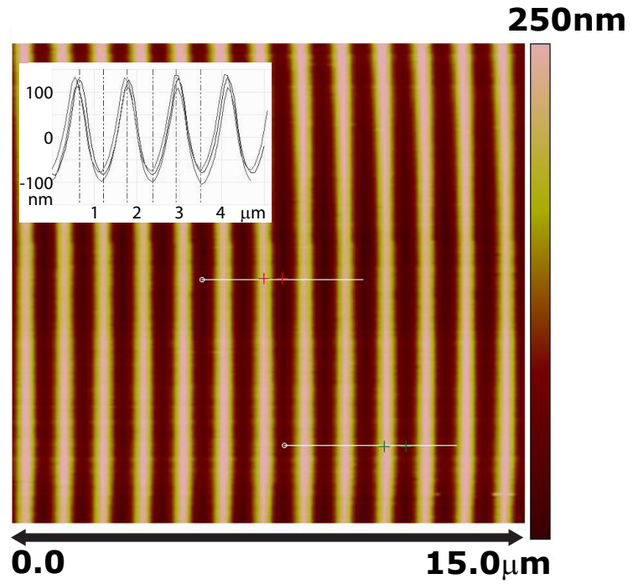

Figure 4. Atomic force microscopy image of 1400nm pitch features patterned on peptide functionalized hydrogels.