Co-culture Separation and Assembly by Nanotopographical Persuasion of Cells

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Statement of Purpose: Surface structuring of implantable devices and cell culture environments provide a significant opportunity to control cellular behavior both *in vivo* and *ex vivo*. Without necessarily attempting to 'mimic' the natural environment, micro- and nano-scale cues can be used to guide cell adhesion, proliferation, migration and differentiation towards a desired outcome[1-3]. Understandably, the impact of such topographical cues on cellular response is not uniform across different cell types. We apply nanopillars in both gradient and arrayed format to selectively guide the behavior of two distinct cell types on a single substrate towards higher order spatial organization, demonstrating enhancement and inhibition of endothelial and fibroblast cell activity – along with variation in co-culture cell ratios with a statistically significant dependence on pillar height. To achieve this goal, we use automatic segmentation of fluorescent image data by machine learning algorithm[4], allowing us to distinguish between cell types without the need for cell type specific labeling.

Methods: Arrays of nanodots were defined on a quartz substrate (Qz) by electron beam lithography and lift off of aluminum. A gradient of plasma polymerized hexane (ppHex) thickness was deposited as a sacrificial etch mask before etching in a standard RIE process, creating a gradient of pillar height across the sample, *Figure 1*.

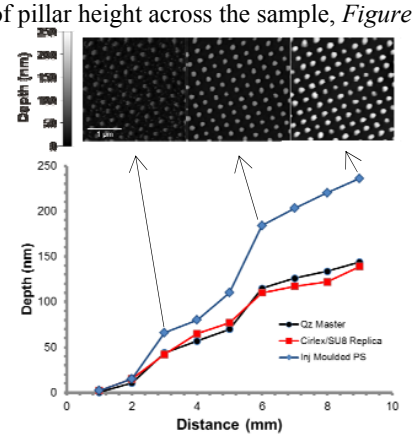

Figure 1: Selected AFM scans of short, medium and tall pillars on a gradient of nanopillar height, replicated in polystyrene by injection molding after fabrication in quartz (Qz)

Co-cultures of fibroblast (hTERT-BJ1) and endothelial (LE2) cells were cultured for up to 96h on nanopillar substrates, before fixation and fluorescent labeling of the cell cytoskeleton & nucleus, or bright field imaging after staining with coomassie blue The CellProfiler software suite was used for image processing.

Results

After initial seeding of the two cell types at an even density across the nanotopographical gradient of pillar height, the ratio of endothelial to fibroblast cells was found to vary over time as a function of the underlying topographical motifs, *Figure 2*. The number of fibroblasts fell steadily with increasing nanopillar height, whilst there was a moderate increase in the abundance of endothelial cells with increasing pillar height. Endothelial cell numbers also fell away at extreme pillar heights, which suggests an optimal height at approximately 75 nm for the induction of increased endothelial proliferation and fibroblast migration away from the structured regions.

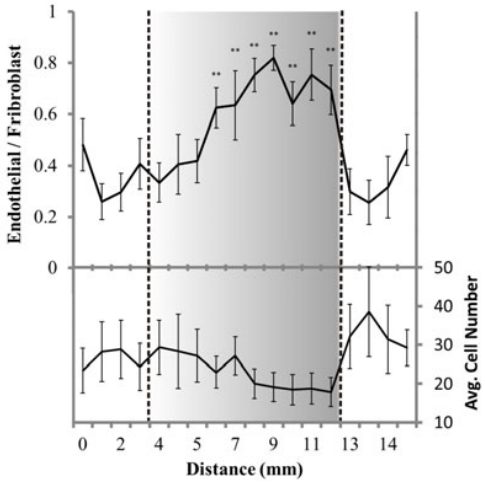

Figure 2: The ratio of endothelial/fibroblast cells rises as nanopillar height increases (from left to right within dashed lines), and is accompanied by an overall reduction in total cell number approaching extreme heights.

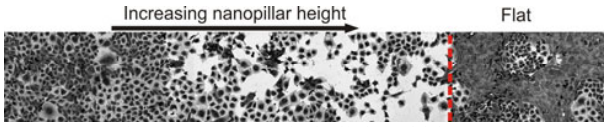

Figure 3: Characteristic endothelial 'cobblestone' formation is apparent on the nanopillar regions, whilst the fibroblast population actively migrates until aggregation on the flat, unstructured region of the substrate.

Using this stark contrast in response exhibited by each cell type, cellular migration and proliferation response can be directed into predefined regions of the sample after initially uniform seeding, with no requirement for chemical manipulation, *Figure 3*.

Conclusions

Interspersed regions of flat and structured surface regions offer the opportunity to guide cell migration and aggregate certain cell types in specific locales – with the aim of inducing super-cellular organization. We have demonstrated the substrate driven segmentation of a co-culture, including optimization of nanopillar height by screening against a novel gradient of pillar heights.

References: [1]Reynolds M. Small, *2012*;**8**(16);2541. [2]Csaderova, L. Small, *2010*;**6**(23);2755. [3]Dalby, M.J. Nature Mater, *2007*;**6**;997. Nat Mat. Jones, T.R. PNAS *2009*;**106**(6);1826