Development and Characterization of a High-Throughput Screening Surface Combining Geometric and Nanotopographical Mechanical Cues to Investigate Cell-Surface Interactions

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Statement of Purpose: Biomaterial surface properties influence many cellular responses such as adhesion[1], migration, and differentiation.[2,3] For example, various mechanical cues have been shown to initiate[2] and enhance[3] differentiation in mesenchymal stromal cells (MSC). However, few current fabrication strategies combine different mechanical cues provided to cells to investigate the synergistic effect of these cues. Our research focuses on designing a high-throughput screening (HTS) surface to combinatorially explore different cues presented to cells with the aim of investigating how different cells such as fibroblasts, endothelial, epithelial, osteosarcoma, and/or MSCs respond to the cues. In this study, we explore the effects of the combination of several key cues presented to cells on 2D biomaterial substrates: i.) various geometric cue shapes/sizes and ii.) different types of underlying nanotopographical features.

Materials and Methods: Nanotopographical patterns (square (sq) and hexagonal (hex) arrayed round pits and square arrayed round pillars (pillars)) were replicated in biocompatible Topas®5013 polymer (Topas Advanced Polymers) by injection molding (Victory 28, Engel GmbH). Adhesive geometric micropatterns overlying the nanotopography were fabricated using Shipley Microposit™ S1818 resist with standard photolithography methods and oxygen plasma treatment to create adhesion patterns on the hydrocarbon surface. Surface chemical composition was determined with X-ray Photoelectron

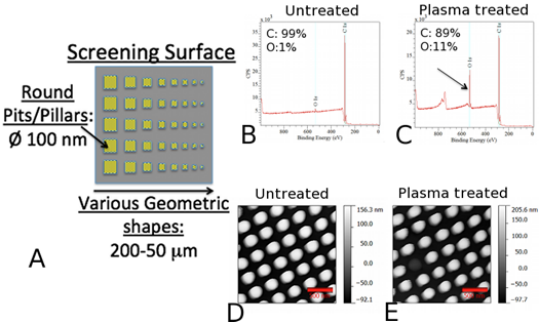

Fig. 1 (a) Depiction of a surface combining geometric cues and nanotopography on a transparent polymer substrate, XPS results before (b) and after (c) O_2 plasma treatment, and AFM measurements before (d) and after (e) O_2 plasma treatment. Scale bar = 500nm

Spectroscopy (XPS), and the integrity of the nanotopographical surface was verified using atomic force microscopy (AFM) and scanning electron microscopy (SEM). hTERT immortalized human primary fibroblasts were screened on the high-throughput combined cue substrates. Cell surface interactions were analyzed through immunohistochemical staining of actin and the focal adhesion (FA) protein vinculin.

Results: Little work has been reported in the field to investigate the combined effects of different static mechanical cues that have previously been shown to illicit cellular responses due to cell-surface interactions. This may be due, in part, to difficulties in successfully combining fabrication techniques required to create nanotopographical features on materials suitable for both photolithography and cell culture. We have successfully engineered a transparent surface which has underlying nanotopography coupled with geometric shape gradients capable of supporting cell culture.

Figure 1a illustrates the screening surface with nanotopography (Ø 100 nm, pitch 300 nm) underlying geometric shape gradients (50 µm-200 µm) in the cell adhesive regions (yellow). Cellular adhesion on the nonadherent Topas surface was achieved by O_2 plasma treatment of the masked surface to introduce adhesive oxygen species (11%; C-O, C-OH) (Fig. 1b,c). The integrity of the underlying nanotopographical features was retained after the plasma treatment (Fig. 1d,e).

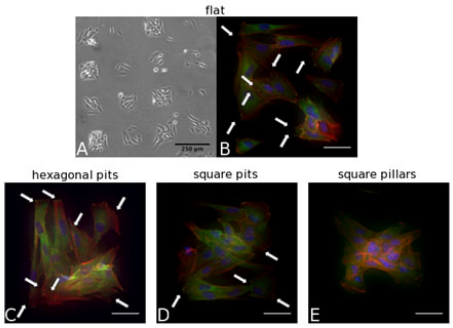

Fig. 2 hTERT cells cultured in square geometric micropatterns on (a,b) flat and underlying nanotopography: (c) hex (d) sq (e) pillars. Blue=nucleus, red=actin, green=vinculin. Arrows highlight FAs. Scale bar=50µm, 100x (a) and 200x (b-e) magnification.

hTERT cells were successfully confined to the geometric micropatterns (Fig.2a). Normal FAs (Fig.2b) were disrupted to varying degrees depending on the underlying nanotopography: slightly on hex, moderately on sq, and severely on pillars (Fig.2c,d,e, respectively).

Conclusions: We demonstrate the successful fabrication of a transparent, HTS surface that combines geometric micropatterns and nanotopographical mechanical cues. We have shown that the mechanical cues of these screening surfaces significantly affect the cytoskeleton and FA complexes of hTERT cells depending on the underlying nanotopography. We are currently exploring the response of hMSCs to the array of combined mechanical cues on the fabricated screening surfaces.

References: [1]Curtis, A.S.G. IEEE T NANOBIOSCI, 2004;**3**:61-65. [2]Dalby, M.J. Nature Mater, 2007;**6**;997-1003. [3]Kilian, K.A. PNAS, 2010;**107**;4872-4877.