Cell Behavior on Collagen-Like Imprinted Nanostructures in Tissue-Culture Polystyrene

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Advances in tissue engineering can only occur as our understanding of the cell-substratum interactions improve. In nature, we can see how cells behave in their natural environments; however, we can't determine the underlying reasons for specific behaviors. Biomimetic materials can be precisely engineered in a cheap and controlled manner to mimic this environment while observing cell behavior in direct response to the controlled material features. With this knowledge, nanostructured biomaterial scaffolds can be designed and controlled to optimize direct cell growth and tissue regeneration.

Most research which seeks to understand these interactions concentrates on 2-D micron scale scaffolds. Realistically, to fully understand the behavior of cells in vivo, one must consider the effect of 3-D nanotopography in a biomimetic environment which more closely mimics the extracellular matrix (ECM). 3-D nanoscaffolds more closely mimic the network of collagen fibers in which cells migrate, align, deform and proliferate in vivo. To fabricate collagen-like scaffolds at the nanoscale, nanoimprint lithography (NIL) is utilized. This technique is suitable because of its' nanoscale resolution, high throughput, flexibility, low cost and ability to fabricate large areas.

Conventional NIL typically patterns spin-coated polymer films on underlying substrates, making it difficult to use in some tissue engineering applications. In direct NIL, tissue-culture polystyrene (TCPS) is directly imprinted with micro and nanostructures. TCPS is a commercially available optimized surface for cell attachment and growth. These structures range in size from 500 nm to 10 μm. Additionally, a second imprint (140 nm wide) was carried out on top of the first lines (500 nm wide) perpendicularly to pose as cross striations which occur naturally on collagen fibers. These structures are shown in Fig. 1. In addition to conventional NIL, a reversal NIL method is also utilized to mimic the ECM cells inhabit. Collagen fibers are generally clustered in groups in nature, creating an environment much different from gratings. For this reason, a second group of structures was created to simulate this clustering effect. Optical lithography and reversal NIL were used to create a "woodpile" structure composed of different layers of 10 μm gratings as shown in Fig. 2. These structures will reveal important information regarding how cells migrate and deform within these clusters.

To investigate the effects of the collagen-like 3D nanoscaffolds on cell behavior, human foreskin fibroblasts were cultured on imprinted substrates. After the cells adhered and spread on the surface, they were fixed and stained with fluorescent phalloidin, which binds to cytoskeletal filaments, and visualized by phase contrast and fluorescence microscopy (Fig. 3). Alignment of the cells was judged by the angle between each cell's longest axis and the direction of the grating. 75% of cells were aligned (within 15°) on the 1 μm grating and 67% were aligned on the 0.5 μm grating. On a flat control surface adjacent to the imprinted surfaces, only 9.5% of cells were aligned with the direction of the adjacent grating, and the distribution of angles was consistent with random orientation of the cells.

The cross striations of the collagen-like fibers are expected to have different cell behavior compared to 2D gratings. With the knowledge gained from this set of experiments, we expect to have a better understanding of how cells respond to nanostructures and react in a biomimetic environment.

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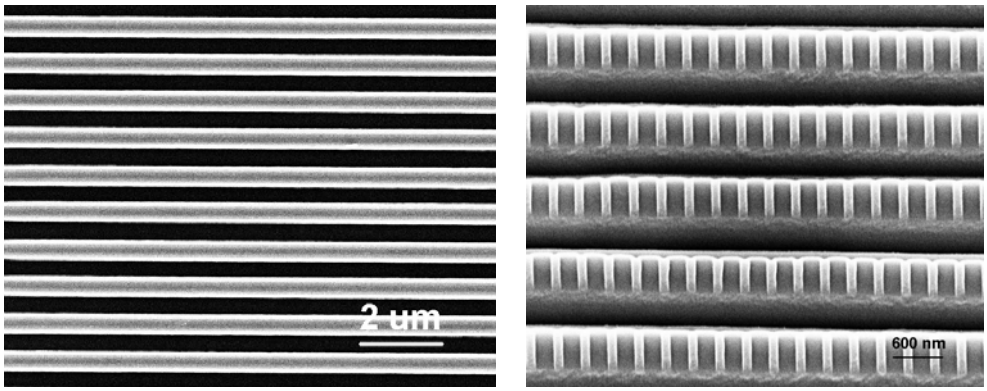


(a) (b)

Fig. 1: Nanoimprinted structures in tissue-culture polystyrene: a) 0.5 μm half-pitch gratings and b) 0.5 μm half-pitch gratings embedded with 140 nm-wide cross-striations fabricated by double direct NIL process to mimic natural collagen fibers

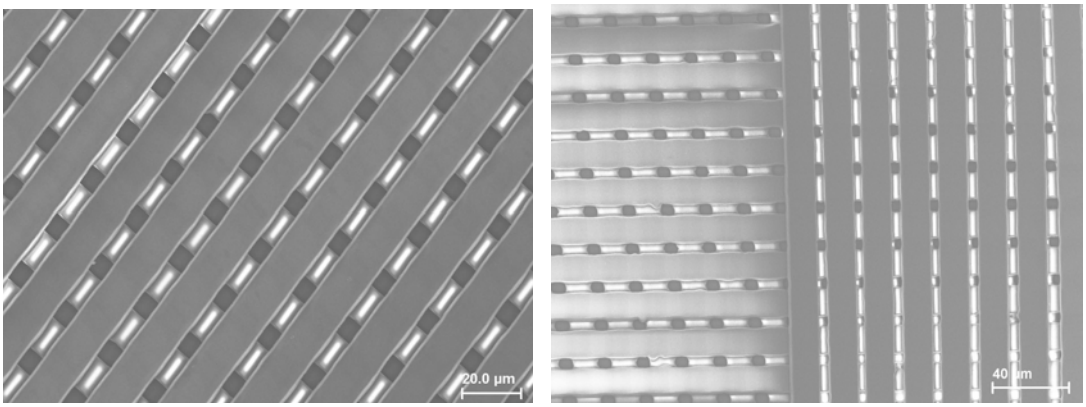
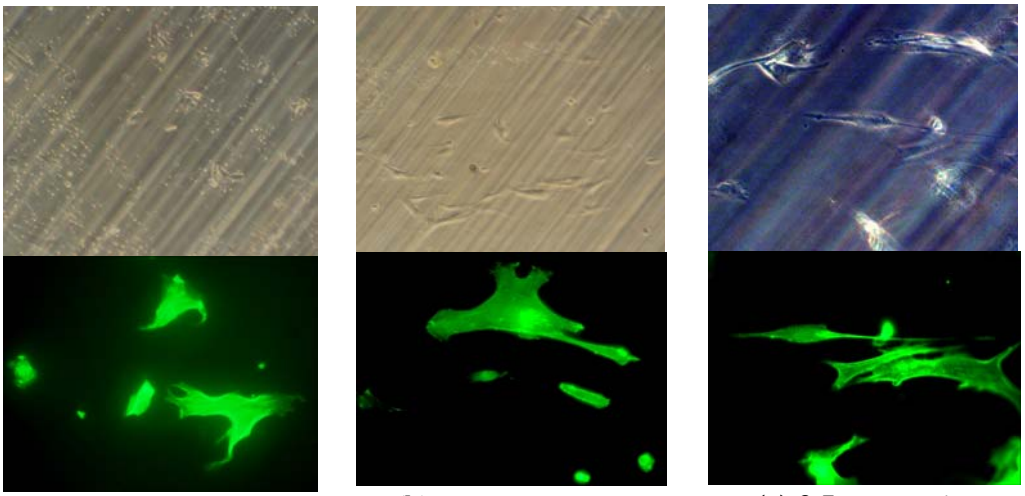


Fig. 2: Woodpile SU8 structures made by optical lithography and reversal NIL. 2 and 3-layer woodpiles are shown consisting of 10 μm wide lines, while more than 6 layers will be used.



(a) non-patterned (b) 1 μm grating (c) 0.5 μm grating

Fig. 3: Phase contrast images (upper) and fluorescence microscopy (lower) of cells on nanoimprinted TCPS. The cells align in the horizontal direction, which is the grating direction. *Note that the strikes in phase contrast images are texture on the back of TCPS. Nanoimprinted gratings that oriented horizontally did not appear in the optical microscope images at low magnifications.*