INTEGRATING BOTTOM-UP AND TOP-DOWN FABRICATION: A HIERARCHICAL APPROACH TO BIOMIMETIC ENVIRONMENTS FOR STEM CELL MANIPULATION

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The combination of microfabrication with molecularly designed self-assembling materials offers the possibility to control biological events from the molecular to the macro scale. This approach could facilitate the development of biomimetic environments with hierarchical organization that are designed for optimal biochemical and physical cellular stimuli. Therefore, we describe ongoing work concerning the integration of bottom-up and top-down fabrication techniques to develop nano- and micro-structures from self-assembling peptide amphiphiles (PA) molecules for selective manipulation of human mesenchymal stem cells (hMSCs).

PA molecules containing specific peptide sequences were designed to self-assemble into well-defined nanofibers that form a highly hydrated gel. The PA comprised the bioactive epitope Arginine-Glycine-Aspartic Acid-Serine (RGDS) to promote cell adhesion and a diacetylene segment for intra-fiber cross-linking with UV irradiation in order to enhance the mechanical properties. The PA materials were synthesized and purified using methods previously described.[1] Microfabrication and soft-lithographic[2] techniques were combined with self-assembling PAs to realize precise topographical patterns made from randomly oriented or aligned nanofiber bundles (~30 nm in diameter) of self-assembled PA molecules. In order to investigate the effect of the PA topographical structures on hMSC behavior, two sets of experiments were conducted. First, PA topographical patterns with randomly oriented nanofibers were used to determine effects on hMSC differentiation into the osteoblastic phenotype. Then, PA structures with aligned PA nanofibers were used to investigate competitive effects between nano- and microtextures on hMSC alignment. Experiments were analyzed using reverse transcription polymerase chain reaction (RT-PCR), immunofluorescent staining, brightfield, confocal, time-lapse, and scanning electron microscopy (SEM).

The developed fabrication techniques realized up to two levels of PA topographical patterns that comprised holes, posts, and channels down to 5 μm in lateral dimensions and up to 10 μm in height. Cell behavior depended on the topographical features on which they were growing. On the hole microtextures, hMSCs expressed a more differentiated phenotype than those growing on smooth (no microtextures) PA substrates as evident by significantly higher levels of Osteopontin (OP) mRNA expression and staining for Core Binding Factor α-1 (CBFα-1) and OP. An even higher degree of topographical control was achieved by controlling nanofiber positioning. On these substrates, hMSCs recognized the aligned nanofiber bundles and tended to align in the direction of nanofiber orientation even in the presence of 20 μm diameter holes. Interestingly, cells growing on the substrates with microchannels that were perpendicular to the direction of the nanofibers aligned preferentially to either the microchannels (45% of the cells) or to the nanofiber bundles (35% of the cells).

By combining top-down and bottom-up fabrication approaches we were able to create, for the first time, hierarchical topographical patterns with well-defined nano- and microtextures made from self-assembling bioactive molecules. These hierarchical structures may be used to create biomimetic environments that selectively guide and control cells through both biochemical and physical cues in a variety of tissue engineering and regenerative medicine applications. Our results demonstrate the capacity of cells to recognize both bioactive nano- and microscale topographies and raise the possibility of using this approach to enhance cell differentiation. Moreover, the developed fabrication techniques may facilitate the use of bioactive, biocompatible, and biodegradable PA molecules as structural materials and potential components of biomedical microsystems and devices.

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