Hierarchical micro-nano-structures for cell adhesion studies

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Introduction

The capacity to fabricate materials exhibiting well-defined features able to selectively interact with biology at cellular and subcellular levels has had tremendous implications in the field of tissue engineering. It is now well established that cell behaviors can be controlled, enhanced, or diminished by interacting with surface topographies of different size scales (1-3). However, the reasons behind these effects are not well understood and motivate the development of materials that facilitate the systematic study of cell-topography interactions. With this in mind, we report two different fabrication processes to build hierarchical structures in a variety of different materials in order to investigate the competitive effects of micro and nanotopographies on cell adhesion, spreading, and morphology.

Materials and Methods

Micro and nanofabrication techniques such as ion beam lithography (FIB), electron beam lithography (EBL), photolithography, and reactive ion etching (RIE) were combined to create micro/nano hierarchical structures on silicon. Two distinct strategies were developed in order to create high resolution surface topographies with the chance to build versatile designs. Then, these structures were transferred to a number of biocompatible polymers including polydimethylsiloxane (PDMS), polymethylmethacrylate (PMMA), low density polyethylene (LDPE), and recombinant elastin-like polypeptides (ELP). PMMA samples consisted on four different patterned areas with microchannels, nanochannels and perpendicular and parallel micro/nanochannels were fabricated in order to determine the competitive and synergetic effect of the micro- and nano-scale topographies in rat mesenchymal stem cells adhesion and morphology.

Results and Discussion

Scanning electron microscopy (SEM) and atomic force microscopy (AFM) observations revealed that hierarchical topographical patterns consisting of perpendicular and parallel micro/nanochannels were fabricated in silicon and then these structures were successfully transferred to the different polymeric materials. Optical, widefield epifluorescence, confocal, and SEM observations revealed that the cells changed their morphology, alignment and elongation, depending on the different surface topographies (Fig. 1). Cell alignment and elongation significantly increased on parallel nano/microchannels (Figs. 1, 2). However, cells did not have a significant preference for micro or nanochannels in perpendicular region (Fig. 2).

Conclusions

We have developed two distinct methods to fabricate hierarchical structures with high resolution and accurate topography control in silicon and biocompatible polymers. Due to the opportunity to interact with biology at both the nano and microscale, these types of hierarchical structures could be used for a variety of applications in tissue engineering and regenerative medicine. Surface topographies with hierarchical features expanding from the nano to the macroscale offer the possibility to synergistically improve the bioactivity of materials and control biological processes.

References

[1] M.J. Dalby, N. Gadegaard, R. Tare, A. Andar, M.O. Riehle, P. Herzyk, C. D. W. Wilkinson, R. O. C. Oreffo, *Nature Materials* 6 (2007) 997–1003
[2] R. J. McMurray, N. Gadegaard, P. M. Tsimbouri, K. V. Burgess, L. E. McNamara, R.Tare, K. Murawski, E.Kingham, R. O. C. Oreffo, M. J. Dalby, Nature Materials 10 (2011) 637–644
[3] A. Mata, L. Hsu, R. Capito, C. Aparicio, K. Henrikson, S. I. Stupp, Soft Matter 5(6) (2009) 1228–1236

Figures

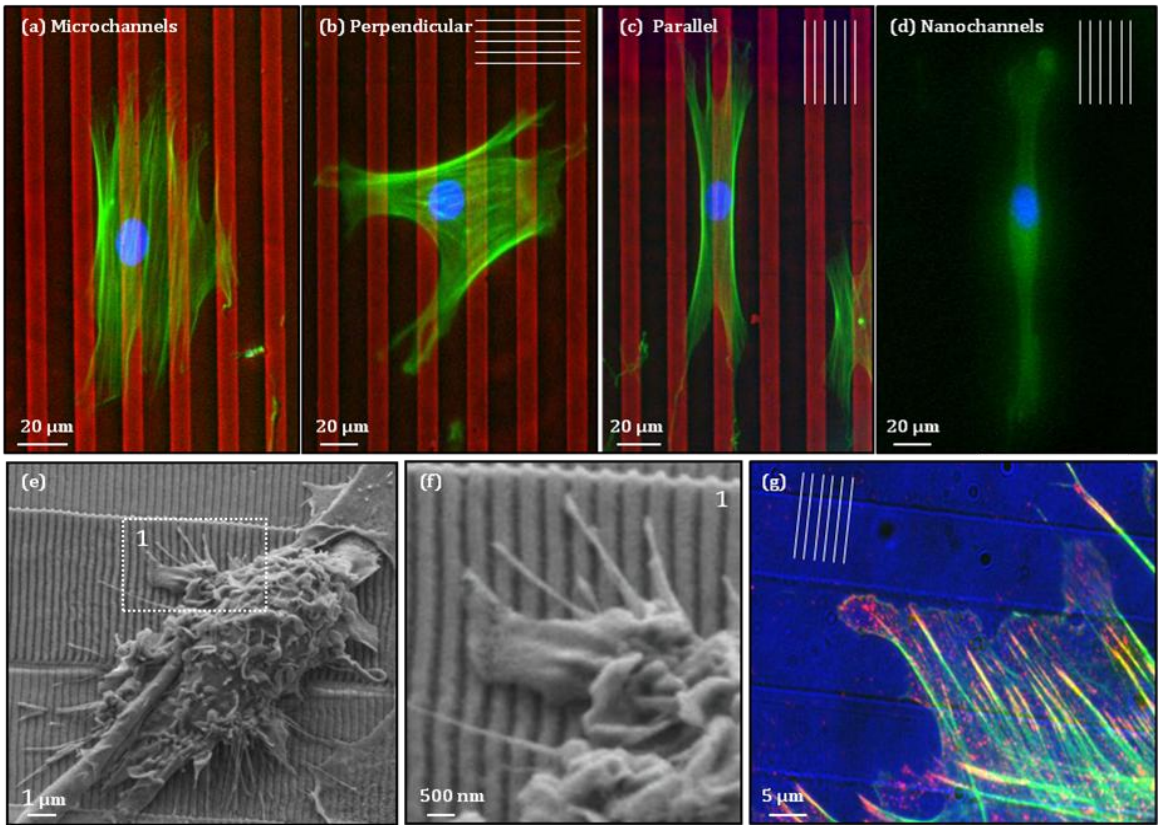

Figure 1. Representative fluorescence images of cells on (a) micro- (b) perpendicular (c) parallel and (d) nano-channels. (e-f) SEM and (g) fluorescence images (red=vinculin, green=actin cytoskeleton) of cells growing on perpendicular channels. Direction of nanochannels is schematically shown by white lines (b-d, g).

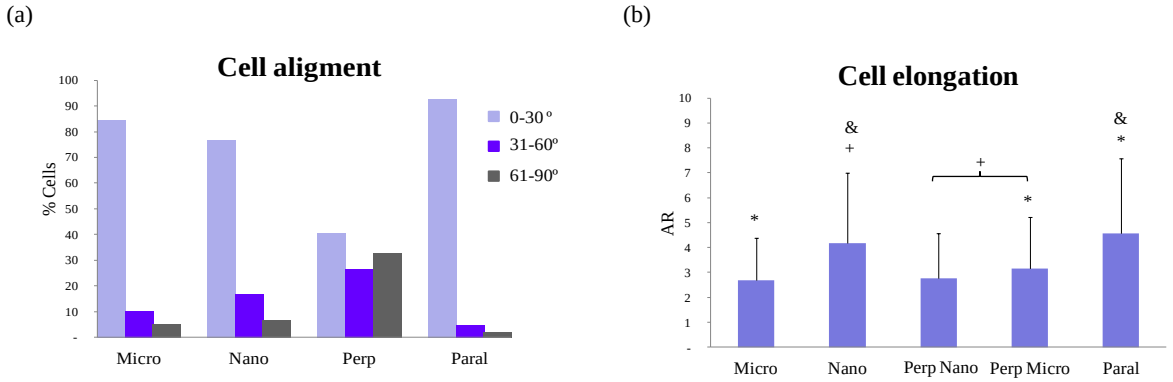

Figure 2. (a) Quantification of cell alignment revealing that cells are aligned preferentially along the micro-, nano- and parallel channels. However, cells sense the competitive effect of the micro- and nano-scale topographies, interacting with both micro- and nano-channels when perpendicular to each other. (b) Quantification of cell elongation revealing that cells sense the synergistic effect of the micro- and nano-topographies on parallel channels. The cells are significantly more elongated on parallel channels compared to the micro- and nano-channels individually.