Fabrication of nano scale topographically structured surfaces using block-copolymer and nanoimprint lithography for cellular response analysis

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A fundamental question in the design of prosthetics for tissue engineering applications is how substrate topography regulates cell behavior. Our previous and ongoing work has focused on defining the effects of topography of native basement membrane relevant length scales on cell behaviors. We have found that the basement membrane underlying a number of tissues contains features with dimensions of 20 to 400 nanometers in size[a]. We fabricated silicon surfaces that contain feature sizes ranging from 400 to 4000 nm in pitch as well as planar regions. We find that nanometer length features affect cell behavior (cell elongation and migration[b], focal-adhesion formation[c], proliferation[d], and adhesion[e]) differently than do micron scale features and planar substrates. These surfaces only begin to probe the biological significant length scale. The aim of the research described here is to enable substrate fabrication to assess cellular behavior over the entire biological range of 10's to 100's of nanometers, requiring development of smaller, yet sill well defined, structured surfaces. Important factors to consider when designing a fabrication process for these substrates include control over the lateral dimension (or pitch), the depth of the features, and the ability to produce a great number of substrates with large patterned areas for statistically robust cell culture assays. Figure 1 highlights the importance of the depth of the features to cellular response: too shallow and the cells do not show behavioral differences due to the lateral scale of topography.

Our approach uses the self-assembly of block copolymers to define controlled lateral feature dimensions with nm accuracy. To obtain substrates with features of appropriate relief (>300 nm), the pattern is (1) transferred into an underlying silicon substrate by selectively removing one of the blocks and using the remaining block as a soft mask for reactive ion etching (RIE), (2) use the block copolymer derived substrate as a master for nanoimprint lithography from which the pattern is transferred into an imprint resist that is spun onto a hard mask coated silicon wafer, (4) etch the pattern into the hard mask material, and (5) etch the pattern into silicon. Figure 2 shows the steps for fabricating the master from block-copolymers (A) and the steps for transferring the pattern into substrates with appreciable depth (B). SEM images of the master are shown in figure 3, and the resulting imprint into resist is shown in figure 4.

(a) G.A. Abrams *et al.*, Cells Tissues Organs **170**, 251 (2002); Cell Tissue Res. **299**, 39 (2000); Cornea **19**, 57 (2000); (b) D.M. Brunette, G.S. Kenner, T.R.L. Gould, J. Dent. Res. **62**, 1045 (1983); R.G. Flemming *et al.*, Biomaterials **20**, 573 (1999); A.I. Teixeira et al., J. Cell Science **116**, 1881 (2003); N.W. Karuri *et al.*, Microsc. Microanal. **11** (2005); (c) A.I. Teixeira *et al.*, Biomaterials **27**, 3945 (2006); (d) S. Liliensiek *et al.*, J. Biomed. Mater. Res. **79**, 185 (2006); (e) N.W. Karuri *et al.*, J. Cell Science **117**, 3153 (2004).

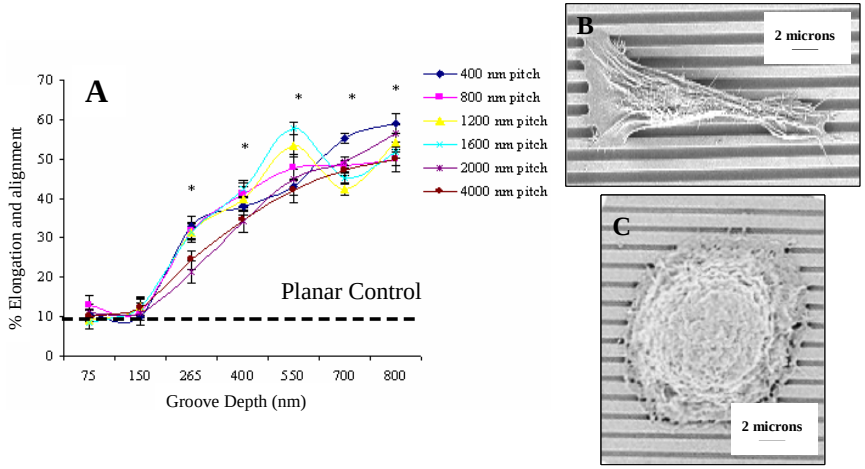

Figure 1. Primary human corneal epithelial cells showed an increased elongation and alignment parallel response to increasing groove depth at all of lateral feature dimensions. (A) % cells aligned and elongated at depths 265 nm and deeper at all lateral pitches were statistically significant (*, p=0.05). (B) Elongated and aligned cell on 265 nm deep features, lateral pitch 1600 nm. Cells did not elongate and align when cultured on sub 75 nm deep grooves, pitch 4000 nm (C).

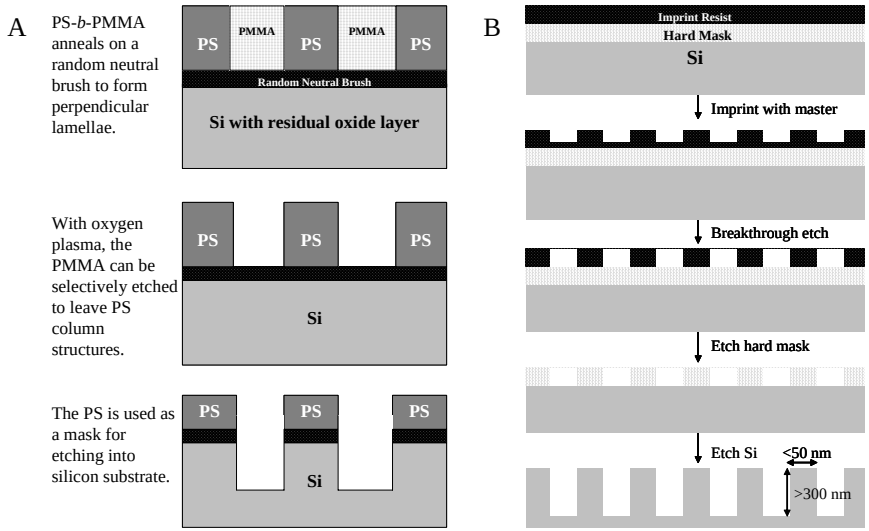

Figure 2. Lithographic plan for patterning surfaces. (A) Making nanoimprinting masters from diblock-copolymer lithography. (B) Using the masters to imprint into a resist, and sequentially etch the residual layer, the hard mask, and the silicon substrate.

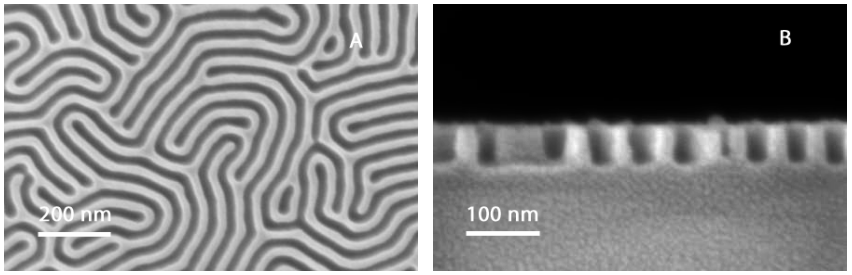

Figure 3. SEM images of randomly oriented perpendicular structures of silicon master for nanoimprint lithography: top-down (A) and cross section (B). The pitch size is 48 nm and depth is 46 nm.

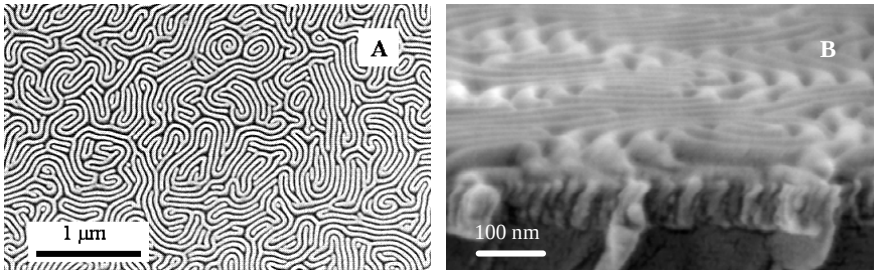

Figure 4. SEM images of imprinted resist: top-down (A) and cross section tilt (B). (B) Shows a layer of chromium under the imprinted resist