Understanding biology through nanostructured interfaces

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Biological function results from the convergence of a complex set of intertwined biochemical and physical processes that occur across multiple length scales. The emergence of techniques for discretely controlling the organization and concentration of signals that influence these processes may help elucidate the role of physical confinement, surface recognition, and molecular transport on the dynamics of biological systems. Such an understanding will shed new light on the development of synthetic strategies for mimicking biological behavior, such as assembly and self-organization, and will open the door for the creation of more effective synthetic interfaces to biological systems. Here, techniques for controlling chemical surface cues using micro- and nanoscale patterning techniques and soluble signal concentrations using nanostructured membranes are demonstrated. Their functional impact on the biologically inspired assembly of silica and the development of both microbial and mammalian cellular interfaces is discussed.

Spatial patterning by chemical lift-off was performed by combining conventional electron beam and photolithographic techniques with the vapor deposition of 3-aminopropyltrimethoxysilane. Patterns were formed in poly(methyl) methacrylate (PMMA) or photo-resist on silicon, and substrates were exposed for two hours to an argon/organosilane vapor. Removal of resist under sonication in acetone left behind chemically functional patterns that were used to either template silica deposition or control the attachment of mammalian 3T3 fibroblasts (Figure 1). Characterization of the functional properties of patterns for both the templating of biomimetic silica and attachment of cells was performed using scanning electron, atomic force and fluorescence microscopies. The techniques demonstrated here are broadly applicable for the development of more specific interfaces, as the amine functional template can be modified using a host of commercially available crosslinkers. This flexibility was demonstrated by functionalizing patterns with a biotin functional polyethylene glycol molecule and subsequent binding of fluorescent avidin.

In addition to patterning chemical cues on interface surfaces, control of local soluble signals is critical for creating more complex interfaces to biological systems. Electron beam lithography and cryogenic silicon etching have been used to create nanoporous silicon membranes. Here we use similar processes to create nanostructured templates from which microfluidic networks having embedded nanostructured elastomer membranes can be created (Figure 2). Such a configuration allows for rapid changes in chemical concentrations to be made within a primary observation microchannel without inducing hydrodynamic flow in that region. Thus repeatable and highly resolved spatiotemporal control of signal concentrations is feasible. We have used this platform, along with phase contrast imaging, to quantify the chemotactic response of microbes to a broad range of chemoattractants.

The combination of surface chemical nanopatterning combined with the control of soluble signals afforded by advanced microfluidics will facilitate the development of comprehensive synthetic interfaces for studying and exploiting biological systems. We anticipate that these techniques will find use in both fundamental studies of biological systems as well as the development of practical tools for *in vitro* toxicity screening, tissue engineering, and biosensor development.

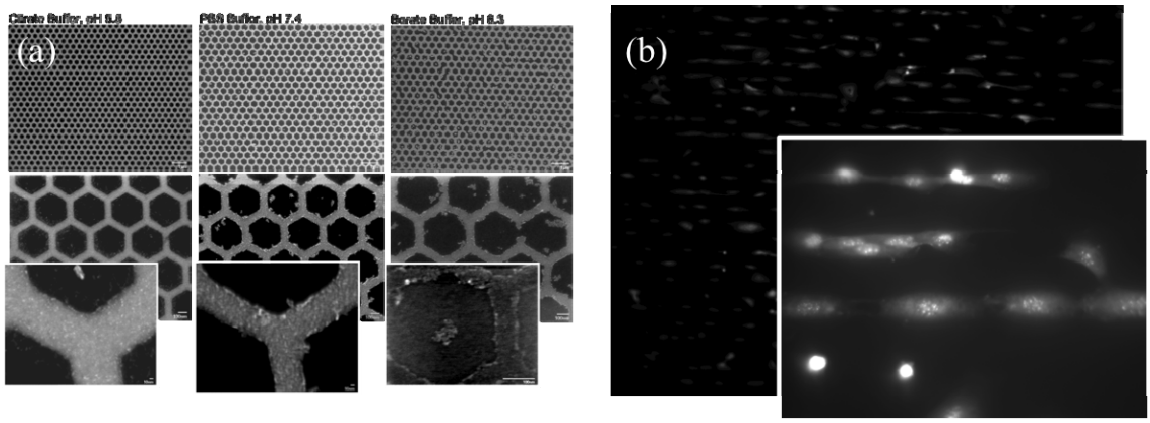

Figure 1 – (a) Patterns of APTMS were used to catalyze the formation of silica under different solution conditions. As expected the pH of the buffer played a signifcant role in the silica morphology, creating larger silica particles under more basic conditions. The line width of patterns shown is nominally 100nm. (b) 25 micron wide lines of APTMS formed using chemical lithography were successfully used to direct the attachment and growth of 3T3 fibroblasts.

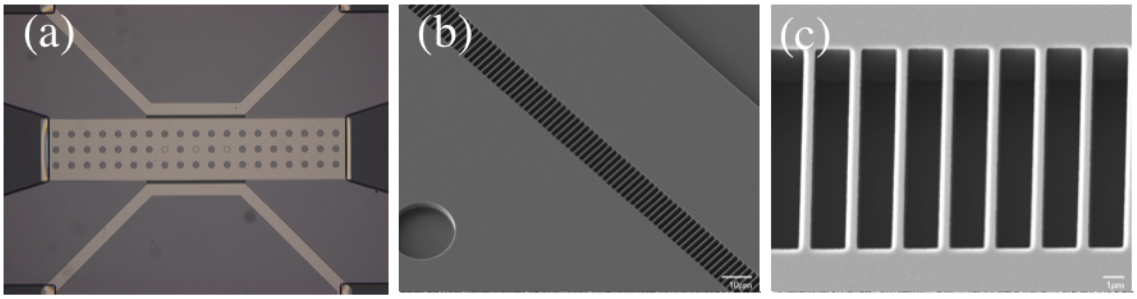

Figure 2 – (a) A silicon and SU-8 master formed using a combination of electron beam and photo- lithography techniques. The channel height of the main chemotaxis chamber can be controlled within tens of nanometers by varying the duration of silicon etching and thickness of the SU-8 photopatternable epoxy resist. (b) The membrane width as well as the density and spacing of nanochannels (c) can be controlled by modifying the electron beam lithography patterns.