Fabrication of Nanoscale Bioarrays for the Study of Cytoskeletal Protein Binding Interactions Using Nano-Imprint Lithography

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Most signaling interactions between functional complexes in biology gain a high degree of specificity through multivalent contacts. This specificity is due to both the chemistry and the spatial arrangement of ligand, receptor and effector domains. In addition, the synergistic function derived from spatial clustering of receptor molecules is very important in inter- and intracellular signaling, and is still very poorly understood. Continued progress in nanofabrication technology now makes it possible to fabricate structures in the size regime of biomolecules, i.e., ~ tens of nanometers and below. Our aim in this work is to implement a system that probes the importance of spatial order in biological systems by using nanofabricated structures to provide multiple protein binding sites with nanometer-scale separations. The nanostructures can be organized into hierarchical arrays in which structural parameters, such as spacing and orientation, are systematically varied.

The nanoscale patterns are used for the study of the dependence of binding of large cytoskeletal proteins on the spatial arrangement of ligands. It has been suggested[1] that the proper ligand spacing can increase binding affinity by orders of magnitude (Fig. 1). The arrays contain metal dots, ~ 5 - 10 nm in diameter, which can be functionalized with linker molecules that specifically interact with individual protein binding sites. These dots can be arranged individually, in pairs, or in more complex patterns based on the structure of the molecules under investigation.

In our work we use nanoimprint lithography for the fabrication of these nano-patterned arrays. The nanoimprint templates are fabricated from diamond-like carbon (DLC) films on silicon, patterned by e-beam lithography with HSQ (negative tone resist), etched with an O_2 plasma and subjected to a plasma fluorination process for an anti-adhesion, anti-wear coating. The nanoarray fabrication process flow (Fig. 2) consists of thermal NIL using PMMA (M_w=25 kg/mol) as a resist, and pattern transfer by electron beam evaporation of AuPd (~ 5 nm) and lift-off. In order to allow the lift-off process for such small features and relatively thin resist layer, a metal hard mask is deposited after NIL by angle evaporation. The hard mask deposition is followed by an O_2 RIE process, which removes the PMMA residue from the bottom of the patterned areas and creates an undercut beneath the hard mask. This process facilitates easy lift-off of metal features in the sub-20 nm size regime. An annealing step at 400 - 500 ^{o}C further reduces the feature size of the lifted off metal and results in highly uniform dots with diameters ~ 5 - 10 nm (Fig. 3).

Biofunctionalization of the pattern with thiolated integrin fragments and attachment to fluorescently labeled proteins is monitored by Total Internal Reflectance Fluorescence (TIRF) Microscopy. Because each ligand is only few nanometers in diameter, we expect only one, but no more than 2 or 3, molecules to attach to each dot. The number of ligands bound to each dot can be quantified using fluorescently labeled peptides. In general, the fluorescence intensity in different parts of the pattern will shed light on the binding and unbinding of the ligands to the protein as a function of their spatial arrangement.

This presentation will describe the fabrication arrays of ultra-small metal features using NIL technology, chemical functionalization and the application of these arrays toward understanding fundamental factors which determine how cells respond to their physical environment.

[1] G. Maheshwari, G. Brown, D. A. Lauffenburger, A. Wells, and L. G. Griffith, .Journal of Cell Science, **113**, 1677 (2000).

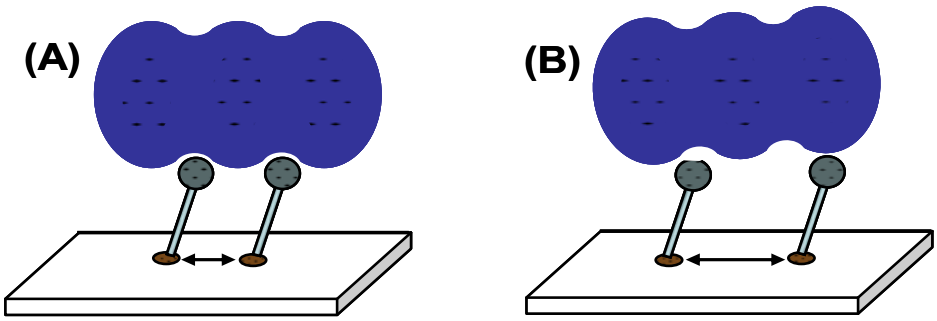

Figure 1. (A) Schematic of a protein binding as a function of binding domain spacing. (A) Ligand spacing matches the domain spacing, implying strong binding. (B) Ligand spacing does not match the domain spacing, implying weaker binding.

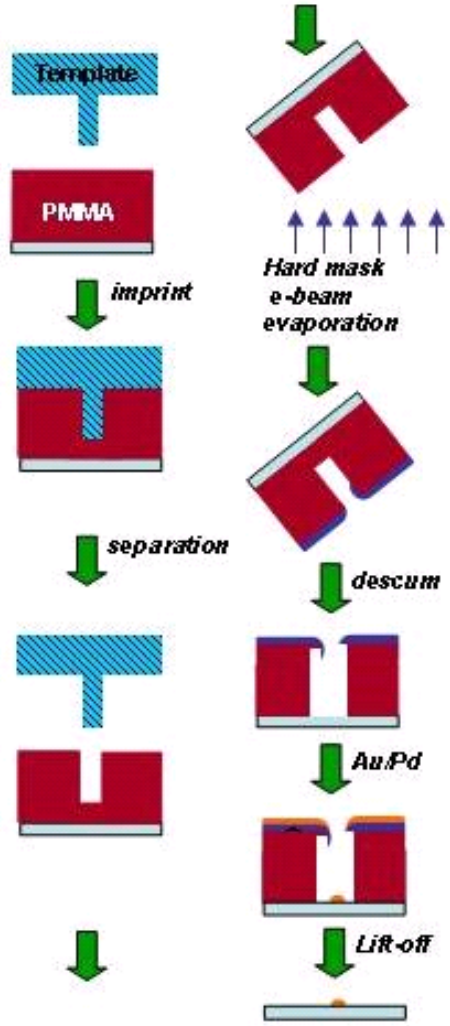

Figure 2. Schematic process flow for the nano-array fabrication.

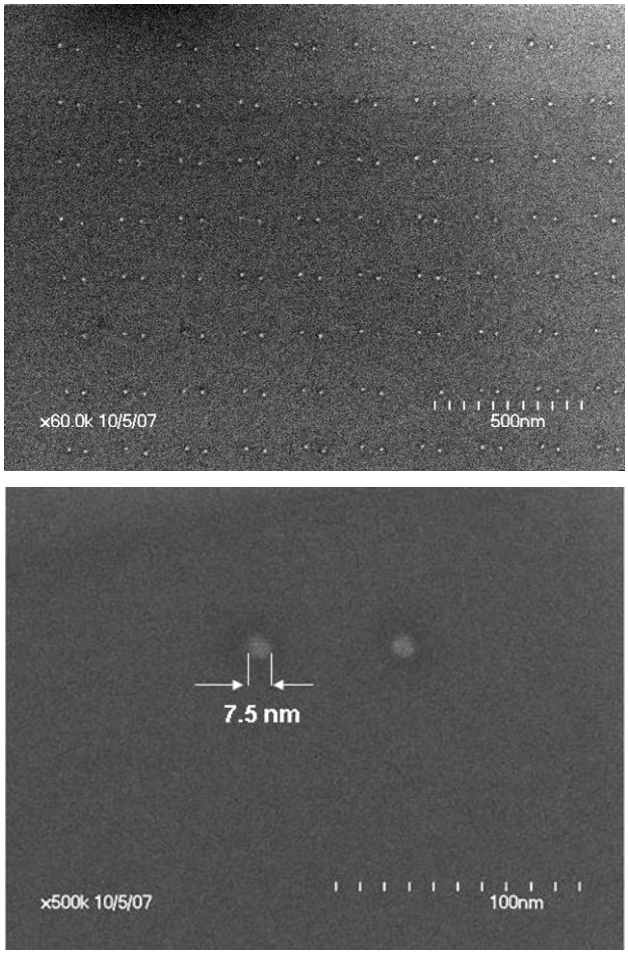

Figure 3. SEM of portion of a nano-dot array consisting of dots pairs, with a distance of 60 nm between the dots in a single pair, and a distance of 200 nm between the pairs. The inter-pair distance is varied from array to array from 20 – 100 nm.

[1] G. Maheshwari, G. Brown, D. A. Lauffenburger, A. Wells, and L. G. Griffith, .Journal of Cell Science, **113**, 1677 (2000).