Nanoscale Patterning of Active Adhesion Proteins

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Statement of Purpose: Immobilization of biomolecules on surfaces can be used in production of novel biomaterials (1) and in analysis of cell-surface interactions (2). The goal of this work is to establish an experimental technique that can be used to produce patterns of proteins that are involved in cell adhesion. The subtractive patterning technique (3) was used to generate nanoscale patterns of the adhesion protein fibronectin (FN). The activity of the patterned protein was investigated using an antibody binding assay and cell binding experiments. This work demonstrates a flexible patterning technique that can be used to produce biologically active environments. Future experiments will lead to an in-depth understanding of the mechanisms that regulate cell adhesion which will be useful in developing biomaterials with specific adhesion protein environments that actively regulate cell adhesion.

Methods: Patterning of proteins was completed using the subtractive patterning technique. A flat elastomer was inked with a monolayer of protein. A nanotemplate was used to produce a pattern of proteins on the elastomer through selective protein transfer in the regions of contact. The nanotemplate was a silicon wafer on which a pattern of etched features was produced using electron beam lithography and inductively coupled plasma etching. Finally, contacting the elastomer to the final substrate transferred the protein pattern (Fig 1). Activity of the protein patterns was assessed using an antibody binding assay. Atomic force microscopy (AFM) was completed on FN surfaces that were treated with the HFN7.1 antibody which is specific to the central integrin-binding adhesion domain of FN (4). A variation in height due to antibody binding to patterned FN indicates specific binding of the antibody to active FN. Cell adhesion surfaces were accomplished by backfilling the protein patterned surface with PLL-g-PEG to produce a non-fouling background.

Results: The subtractive patterning technique was used to print full length fibronectin onto silicon and glass substrates. A range of pattern sizes from micron size to as small as 250 nm were achieved (Fig 1). In order to assess the activity of the patterned fibronectin, an antibody binding assay was used. The HFN7.1 antibody is specific to the central adhesion domain of FN. In the AFM images to the left, the light regions are patterns of FN and the dark background is the silicon substrate. AFM height measurements reveal a height of approximately 6 nm which is in agreement with published values for a monolayer of protein. The antibody binding assay is completed by treating a fibronectin-patterned substrate with the HFN7.1 antibody. If the fibronectin is still active, antibody will bind to the fibronectin patterns and adsorb to the silicon background producing a height difference that can be measured by AFM. The height difference in the image on the right between the patterned area and the background verifies the binding of the antibody. Note that different FN samples were used for the images presented here.

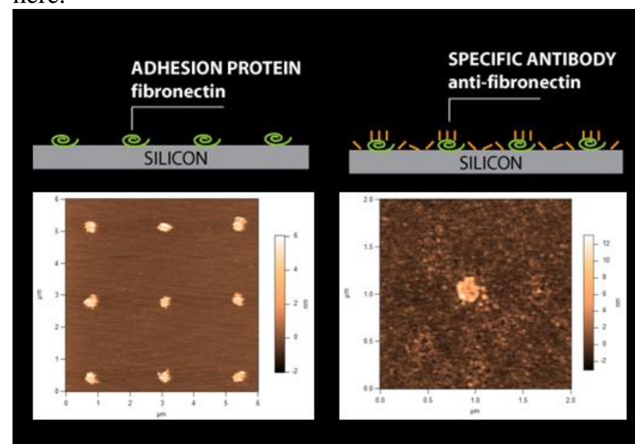

Figure 1. Atomic force microscopy images from an antibody binding assay verify activity of nanoscale patterns of cell adhesion proteins.

Conclusions: These results demonstrate the applicability of the subtractive patterning technique to generation of biologically active surfaces. Patterns of adhesion proteins have been produced in which nanoscale feature sizes and spacing was achieved on silicon and glass. The patterned fibronectin was shown to remain active throughout the patterning process. This method provides the flexibility necessary to produce a wide variety of patterns of proteins that are applicable to studies of biological systems. Future experiments will use the subtractive patterning technique to examine the role of nanoscale organization of proteins in the generation of adhesive cellular forces.

References:

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