Patterning Proteins on Glass and Silicon Substrates

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Abstract:

We have developed two bio-assays in which molecular analytes (streptavidin and anti rabbit IgG) are detected upon binding to patterns of BSA-biotin and rabbit IgG on a glass substrate. The protein pattern was created by microcontact printing with PDMS stamps. We further aimed to develop a microcontact printing process that would enable us to pattern both BSA-biotin and rabbit IgG in an aligned fashion. The success of the aligned microcontact printing process was examined by performing the binding assays with quantum dot conjugates of streptavidin and anti rabbit IgG. Qualitative data analysis was performed by means of optical and fluorescence microscopy.

The experiments showed that both proteins could be patterned and that both binding assays were successful when performed on separate substrates. However, when trying to create bi-arrays on a single substrate, the protein printed first was lifted off in the second printing step.

Introduction:

Microcontact printing has been used by researchers to pattern proteins on silicon and glass substrates. Printed protein patterns are utilized in applications such as biosensors and guided cell growth [1]. In this study we worked towards developing a protocol that would enable us to pattern multiple proteins in an aligned fashion. Multiple aligned protein patterns on the micrometer scale would enhance biosensors by allowing them to test for multiple analytes in one test. Projects that aim at guided growth of cells would also benefit from patterning multiple extracellular matrix proteins on the same substrate.

To achieve alignment of biotin and rabbit IgG we used alignment marks on the substrate as well as on the two PDMS stamps. During microcontact printing we aligned the first stamp with alignment marks on the substrate. The same marks were used to align the second stamp, which was designed with pattern geometries different from the first stamp. The success of the aligned microcontact printing was tested by conducting two binding assays with FITC or quantum dot conjugates of streptavidin and anti rabbit IgG. The use of quantum dot conjugates together with appropriate filters allowed us to visualize both types of bound molecules at the same time [2].

Experimental Procedure

PDMS Stamps:

We first fabricated the desired patterns on two silicon wafers using contact photolithography and deep reactive ion etching. The two components of PDMS (Sylgard 184, Dow Corning Inc.) were mixed in a ratio of 10:1 by volume and were then cast on the surface of the two wafers. After heating to 60°C for one hour, the PDMS was removed from the wafers. The substrates were prepared via a lift off process with two alignment marks in gold.

Microcontact Printing of Proteins:

We coated the PDMS stamps with solutions of BSA-biotin or purified rabbit IgG (1 mg/mL in phosphate buffer, pH 7.0) and let them sit to dry at room

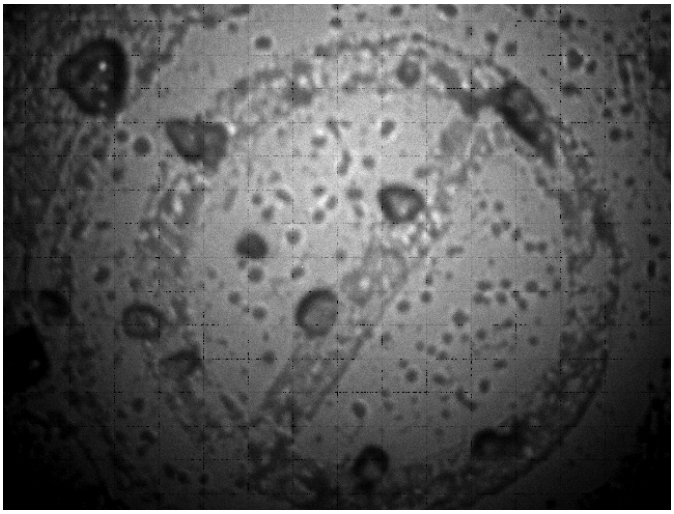

Figure 1: Biotin BSA/ Streptavidin quantum dots patterned on a silicon wafer.

temperature. The stamps were aligned to the marks on the substrate and pressed against the surface. After printing BSA-biotin, rabbit IgG was printed as well on the same substrate.

Bio-Assay:

The surface of the wafer was blocked with a 6% (w/v) BSA solution for 15 minutes. It was washed with phosphate buffer (pH 7.0) and subsequently anti rabbit quantum dot conjugates (maple red, Antibodies Inc.) were incubated on the surface for 15 minutes. The surface was then washed with phosphate buffer containing 0.40% tween for 5 minutes followed by a final rinse with PBS buffer. The same procedure was followed using streptavidin quantum dot conjugates (lake placid blue, Antibodies Inc.). The wafers were the examined with a fluorescence microscope.

Results and Discussion:

On silicon or quartz wafers, the biotin/streptavidin assay produced a distinct fluorescent pattern according to the pattern printed by microcontact printing (see Figure 1). We obtained the same result for the second assay with rabbit IgG/anti rabbit IgG (see Figure 2). These results confirm that the microcontact printing does not affect, in a significant way, the binding capabilities of the proteins printed. For both assays the conditions were sufficiently stringent to produce little unspecific binding of the analyte to the substrate. However, when we performed aligned microcontact printing and the two assays combined, we did not achieve a product of two-colored patterns. One reason for this result may be that the first protein was lifted off from the substrate when the second protein was printed.

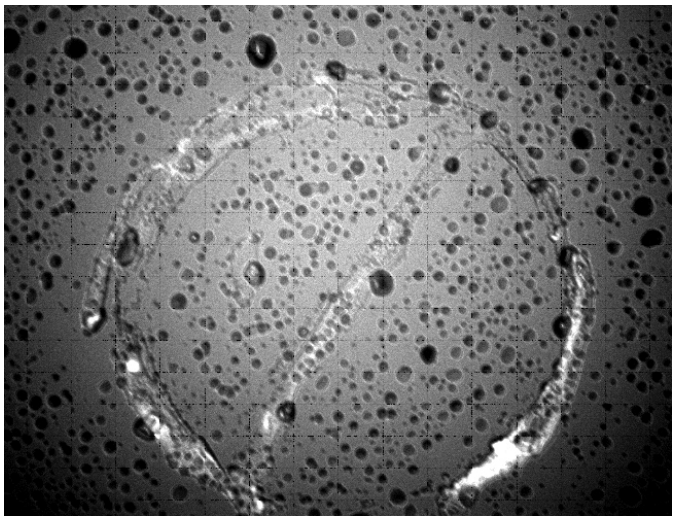

Figure 2: Rabbit IgG / goat anti-rabbit IgG quantum dots patterned on a silicon wafer.

When combining the two assays we also observed an increase in non-specific binding. This may be due to residues left on the wafer from the PDMS stamp.

Conclusion:

In conclusion we found that both microcontact printing procedures and assay protocols worked when performed separate from each other. Though able to produce a single array the method was ineffective in producing a bi-array. Future work needs to be done to examine whether protein is lifted off from the substrate during the second printing process.

Acknowledgements:

NSF, NNIN, and Cornell NanoScale Facility and staff. Special thanks to the Intel Foundation for funding this project.

References:

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[2] G. McDonald, J. Naydeau, K. Nealson, M. Storrie-Lombardi, and R. Bhartia. Fluorescent Quantum dots for Biological Labeling. NASA Tech Briefs. October 2003. Vol. 27, No. 10.