Protein patterning by thermal nanoimprint lithography and NH_3-plasma functionalization of polystyrene

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The ability to spatially orient and anchor proteins on sub-micro to nanometric sized areas has become a major challenge for the development of bioengineered surfaces. The driving force for the ongoing advances concerning methods for biomolecule array fabrication is their prominent application in a variety of fields, including biomedical diagnostics, DNA analysis, drug discovery and environmental monitoring. Moreover, many applications of biomolecule patterns can be enhanced by improving the resolution of the protein features. Smaller feature sizes enable, for example, the fabrication of high density protein arrays for biosensors or proteomic screening, or facilitate studies of cellular interactions with small precisely located clusters of extracellular matrix proteins. Several patterning strategies have been developed to produce biologically relevant patterns. Among them, a major advantage of nanoimprint lithography (NIL) is that the feature size can be reduced to the nanoscale while still retaining high throughput and reproducibility. Most previous work in protein patterning by NIL [1-4] relied on using a sacrificial imprinted polymer to create a pattern of specific protein binding sites. The present work shows a new approach: patterned polystyrene (PS) is functionalized with amino groups incorporated via an NH_3/N_2 plasma treatment [5, 6]; a biotin linker covalently bound to the formed amino groups is used to bind streptavidin (SAv) onto the PS structures. The SAv is used as a versatile anchor for biotinylated functional proteins, as demonstrated in a sandwich-type immunoassay (Fig. 1).

The imprintings were carried out at 180 ºC using a stamp with five different grating periods (ranging from 3.3 to 11.3 μm and 270 nm in depth) on 4" Si wafers having thermally grown SiO_2 on top. The residual layer was removed under an O_2 plasma. The ammonia plasma treatment was performed for 1 min at 20 W in a 4:10 mixture of NH_3 and N_2. X-ray photoelectron spectroscopic (XPS) measurements confirmed the formation of surface amino groups. SAv was immobilized via a sulfo-succinimidyl-biotin derivative linked to the amino groups. To increase the protein binding selectivity between the functionalized PS and the SiO_2 support, treatments of the samples with fluorosilanes and addition of a detergent (Tween 20) to the solutions proved to be most efficient. Fig. 2 includes representative micrographs showing the high affinity to the functionalized PS and the low background fluorescence signal. In order to demonstrate the anchoring capabilities of the immobilized SAv the fluorescence immunoassay depicted in Fig. 1 was performed. First a biotinylated anti-rabbit-IgG antibody is bound. After incubation of a test sample with the analyte solution, bound rabbit-IgG is detected with a second, fluorescence-labelled antibody. Fig. 3 shows the concentration dependence of the recorded signal, yielding a detection limit at about 50 ng/ml.

The presented work shows the great potential of NH_3-plasma functionalized PS for the fabrication of biofunctional structured surfaces, e.g. based on biotin-SAv binding and antigen-antibody recognition.

Acknowledgments
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Figures:

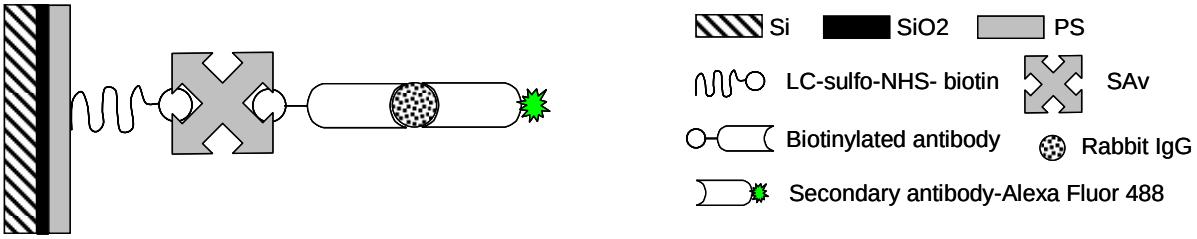
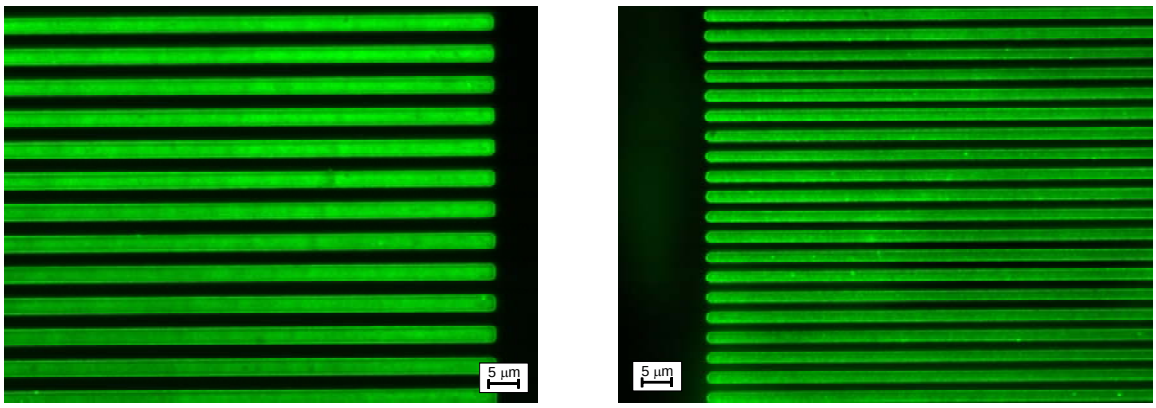


Figure 1. Scheme for the immunoassay aiming at detecting the presence of rabbit IgG in a solution.



a) b)

Figure 2. Fluorescence micrographs of patterned samples after NH_3 plasma treatment and immobilization of fluorescent labeled protein: a) Period 5.1 µm; b) Period 3.3 µm.

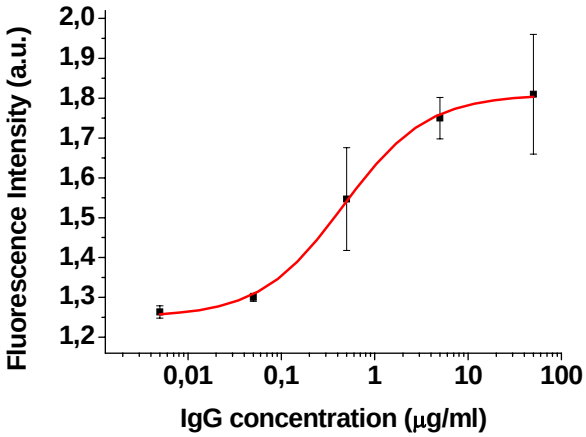


Figure 3. Immunoassay performed on NH_3-plasma functionalized polystyrene: Fluorescence signal with increasing IgG concentration.