PROTEIN PATTERNING ON THE MICRO AND NANOSCALE BY THERMAL NANOIMPRINT LITHOGRAPHY ON A NEW FUNCTIONALIZED COPOLYMER

S. Merino[a], *A. Retolaza*[a], *V. Trabadelo*[a], *A. Cruz*[b], *P. Heredia*[b], *J.A.Alduncín*[c], *D. Mecerreyes*[c], *I. Fernández-Cuesta*[d], *X. Borrisé*[d], *F. Pérez-Murano*[d]

[a] *Fundación Tekniker. Avda. Otaola, 20. 20600 Eibar. Spain*
e-mail: smerino@tekniker.es
[b] *Centro Tecnológico Gaiker. Parque Tecnológico Zamudio. Bizkaia.Spain*
[c]*Cidetec. Centro de Tecnologías Electroquímicas. San Sebastián. Guipuzkoa. Spain*
[d]*Centro Nacional de Microelectrónica, CNM-IMB. Barcelona. Spain*
E-mail: smerino@tekniker.es

The ability to immobilize proteins on sub-micro to nanometric sized areas has become a major challenge for the development of bioengineered surfaces. The ongoing technological advances are partially driven by the aim for broadening the understanding of a variety of surface mediated biological recognition events. Many applications of patterned biomolecules 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. A major advantage of nanoimprint lithography (NIL) is that the feature size can be reduced to the nanoscale to create high density arrays, or to control placement of individual proteins, while still retaining high throughput and reproducibility. NIL was used for protein patterning combining high resolution and high density of proteins [1, 2, 3]. L.J. Guo et al [1] developed a technique based on NIL and fluoropolymer surface passivation to avoid protein adsorption and an aminosilane passivation to form a covalent layer with biotin, exploiting later the specificity of the biotin/streptavidin linkage. M. Textor et al. [2] developed a technique for protein patterning combining NIL and molecular assembly patterning by lift-off. H. Schift et al [3] used NIL and aminosilane deposition followed by lift-off of the sacrificial printable polymer to get streptavidin patterning at the nanoscale. However all these three processes described require the use of a sacrificial printable polymer and vapour deposition of aminosilane, which lead to make the process more complex. The present work shows a new approach: a new bio-functionalized copolymer based on 80% benzyl methacrylate and 20% succinimydil methacrylate is used as a printable polymer with a great affinity to proteins through the covalent binding between the succinimydil group and the amino groups of the proteins. It has been used to develop a patterning in the micro and nanoscale of streptavidin protein. Following this approach an enzymatic sandwich immunoassay has been proposed to detect the presence of rabbit IgG protein as analyte. Besides an in situ sandwich, fluorescence based immunoassay has been developed in order to perform specific detection of the analyte using the patterning of the surface.

Stamps with five different gratings in the microscale (ranging from 3.3 µm to 11.3 µm periods and equal line width and space) and in the nanoscale (200 nm lines width spaced some micrometers) were fabricated by UV-Lithography and e-beam Lithography respectively and etched by a combined SF_6/C_4F_8 plasma to reach 270 nm in depth. The stamps were imprinted on silicon substrates coated with a 270 nm thick film of the functionalized polymer at 160ºC (glass transition temperature of 86ºC) and the residual layer was etched by an O_2 plasma. An antiadhesive coating was applied by evaporation of tridecafluoro-(1,1,2,2)-tetrahydrooctyl-trichlorosilane (F_{13}-TCS) to avoid protein adsorption on the silicon areas. These substrates were soaked in a solution of streptavidin (small concentrations of BSA protein were added to avoid non-specific binding) containing the enzyme HRP and streptavidin marked with the fluorescent label Alexa Fluor 488 for ELISA type tests and fluorescence detection

respectively. Figure 1 includes fluorescence detection showing the streptavidin protein patterning on the micro and nanoscale and the low background fluorescence signal.

With the aim of showing the biological functionality of the streptavidin protein once it is bound to the polymer, an immunoassay is proposed to detect the presence of rabbit IgG protein. Different dilutions of rabbit IgG concentration of a stock of 1 mg/ml were used in microstructured samples for fluorescent detection of the fluorescence-labelled antibody specific for IgG, aiming to establish a correlation between IgG concentration and fluorescent signal intensity. The stock was diluted up to 1:5000 (200 ng/ml) in volume and a photomultiplier was used to detect the light emitted by the fluorescent label in order to show the threshold concentration of the IgG that can be detected. A sample without analyte was used as negative control. Figure 2 shows the average values measured of the fluorescence intensity and from these data it can be concluded that the threshold detection is better than 200 ng/ml.

References:

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[2] D. Falconnet, D. Pasqui, S. Park, R. Eckert, H. Schift, J. Gobrecht, R. Barbucci, M. Textor. Nanoletters 4, **10** (2004) 1909.
[3] S. Park, S. Saxer, C. Padeste, H.H. Solak, J. Gobrecht, H. Schift. Microelectroning Engineering **78** (2005) 682.

Figures:

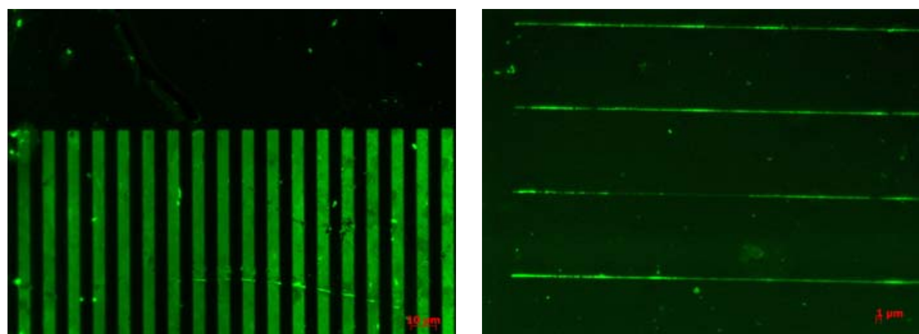

Figure 1. Fluorescence micrographs of patterned samples for a streptavidin concentration of 20 μg/ml containing 0,05 μg/ml of BSA protein. a) Grating with 10 μm period and equal lines and spaces. b) 200 nm width lines.

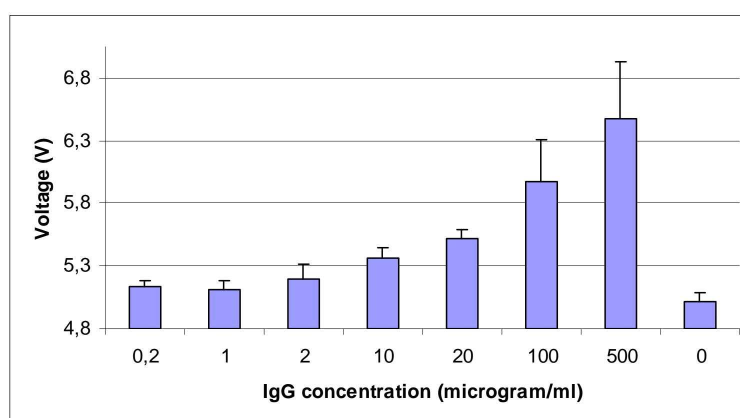

Figure 2. Averaged fluorescent signal measurements as a function of the concentration of the rabbit IgG protein.