PROTEIN PATTERNING BY THERMAL NANOIMPRINT LITHOGRAPHY ON FUNCTIONALIZED POLYMERS

A. Retolaza[a], S. Merino[*,a], V. Trabadelo[a,d], P. Heredia[b], C. Morales[b], J.A. Alduncín[c], D. Mecerreyes[c], H. Schift[d], C. Padeste[d]

[a]Fundación Tekniker. 20600 Eibar. Guipuzkoa. Spain.

[b] Gaiker. Parque Tecnológico, 48170 Zamudio. Bizkaia. Spain.

[c] CIDETEC, Centre for Electrochemical Tech., 20009 Donostia. Guipuzkoa. Spain.

[d]PSI, Paul Scherrer Institut, 5232 Villigen, Switzerland

[*]Corresponding author: 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. Most previous work in protein patterning by NIL relied on combining NIL and molecular self assembly by lift-off [1], NIL and lift-off with fluorinated mono and trichlorosilanes [2] or NIL and thin passivating layer of CFx [3]. All these processes use a sacrificial imprinted polymer and a treatment with aminosilanes so as to bind the biotin and get the hydrophilic/hydrophobic chemical contrast on the substrate surface before putting on the streptavidin (SAV). The work presented relies on two types of functionalized polymers: a new biofunctionalized polymethacrylate copolymer with 80% benzyl methacrylate and 20% succinimydil methacrylate, which shows a great affinity to SAV and it allows to bind the protein directly on the imprinted polymer; and polystyrene (PS) with amino groups incorporated via an NH_3/N_2 plasma treatment [4], which can bind directly biotin without the need of any aminosilane.

The imprintings on the functionalized polymethacrylate were carried out using a stamp with five different grating periods ranging from 3.3 to 11.3 μm, 20 mm long and 270 nm in depth. The polymer was imprinted at 160 ºC applying 20 kN force on 4"wafer and demolded at 35 ºC. The stamp used for the PS consisted of a 10 μm period orthogonal array with 5 μm square 700 nm high pillars. The polymer was imprinted at 180 ºC applying 15 kN on 4"wafer and demolded at 80 ºC. From this point on the experimental procedure was different depending on the polymer, as it is shown in figure 1.

In the first approach an antiadhesive coating was applied to the polymer by evaporation of tridecafluoro-(1,1,2,2)-tetrahydrooctyl-trichlorosilane (F_{13}-TCS). Prior to the fluorescence detection, and in order to study the affinity between the functionalized polymer, the antiadhesive layer coating and SAV, blank small samples coated with the polymer and antiadhesive layer were analysed by ELISA technique. Different SAV-HRP concentrations (0-10 μg/ml) were solved in different buffer solutions at different pHs together with 0.01 mg/ml of BSA. The samples were oxidised under tetramethylbenzidina substrate and the colour change was detected at 450 nm. It led to know that if BSA was added to SAV solutions solved in Carbonate-Bicarbonate buffer it avoided unspecific binding and the selectivity between functionalized polymer and F_{13}-TCS was maximum. Once the process conditions were optimized, a fluorescence labelled SAV (Tetramethyl rhodamine, Molecular Probes) was incubated on the samples.

In the second approach, the ammonia plasma treatment for the PS was performed for 1 min at 20 W in a PECVD PlasmaLab 80 Plus. The NH_3 and N_2 flows were 40 sccm and 100 sccm respectively. Then the biotin and subsequent SAV incubations were carried out. In this case no blocking agents (F_{13}-TCS or BSA) were used to avoid the unspecific binding of the SAV. A fluorescence labelled SAV (Alexa-fluor® 488 conjugated, Molecular Probes) was put on the samples. The specimens after immobilization of SAV were assessed using a fluorescence microscope (Zeiss, Axioimager) equipped with a monochrome CCD camera (Zeiss, AxioCam MRm). Figure 2 includes two representative fluorescence images that show the high affinity of SAV to the imprinted functionalized polymers. The work carried out can be easily extended to patterned surfaces under 100 nm and it will allow patterning any biotinylated protein bonded to SAV in an easier way than described previously while keeping the suitability for high throughput protein patterning.

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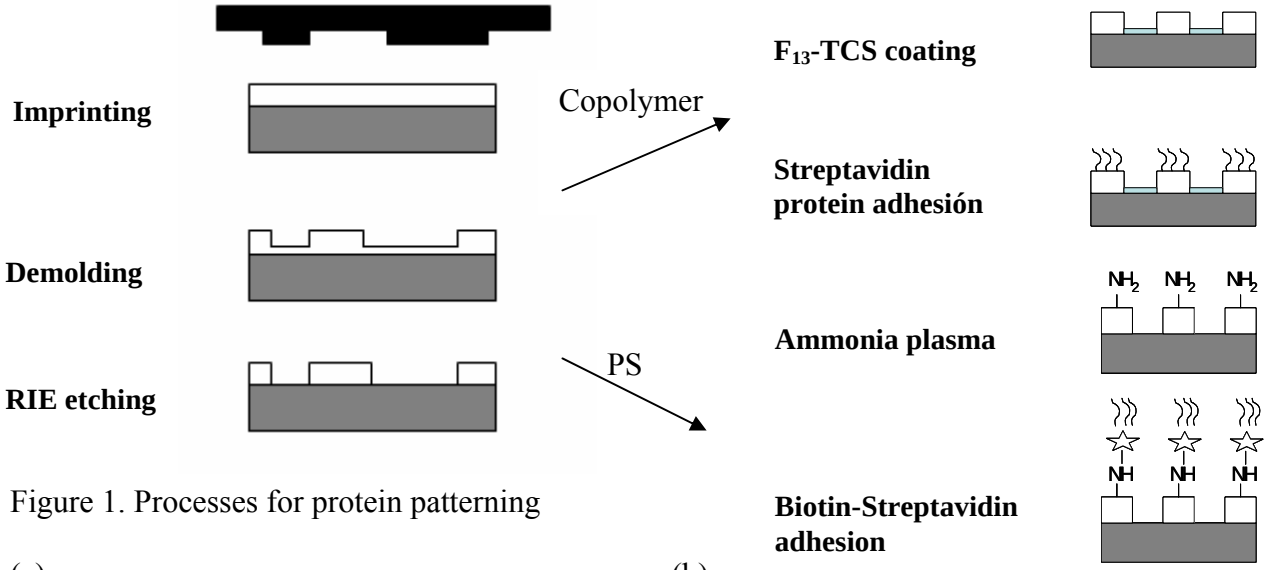

Figure 1. Processes for protein patterning

(a) (b)

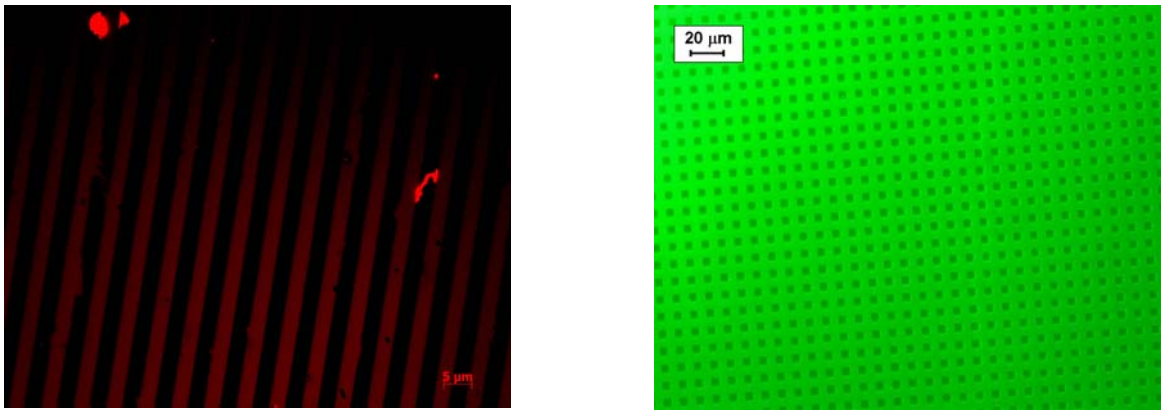

Figure 2. Fluorescence images for (a) first approach (3.3 µm lines) and (b) second approach (10 µm period orthogonal array with 5 µm square holes).