Functional nano-structured surfaces for protein based sensors.

A.Valsesia, , T. Meziani, P.Colpo, M.Lejeune, G. Ceccone, F.Rossi

European Commission Joint research Centre,
Institute for Health and Consumer Protection,
21020 Ispra (Varese), Italy

ABSTRACT

The reduction of the typical length scale in the creation of patterned surfaces is of high interest in the field of biosensor and bio-interacting materials. During the last few years several researchers explored the influence of the nano-structured materials on the protein adsorption or on the cell adhesion assays. The technological difficulty consists in the creation of nano-structures with controlled physico-chemical and geometrical properties, by exploiting the current nano-fabrication techniques, which are potentially compatible with industrial scaling. Several techniques of generation of nanostructured surfaces with chemical contrast for biosensor applications have been developed. In this work is described the fabrication process and characterization of Poly Acrylic Acid nano-domes and gold nano-wells using colloidal lithography

Keywords: colloidal lithography, surface functionalization

1 INTRODUCTION

Patterning of surface with active and non-active spots at sub-micron level is one of the main issues for the development of protein and cell based sensors for drug screening application. The vision is that nano-patterned surfaces with high chemical contrast may allow the triggering of specific interactions exclusively and therefore improve drastically the signal to noise ratio of the bio-analytical devices. Many studies have been undertaken to develop reliable method of chemical nanopatterning and to study the effect of the nano-structured materials on the protein adsorption or on the cell adhesion. Several approaches have been used successfully but many issues still to be addressed. On one hand, the excellent results obtained by using a bottom-up approach (molecular assembly, auto-nano-fabrication) are now far to be scaled to high-throughput systems. On the other hand, top-down approach is often too expensive and time consuming (e.g. electron beam lithography) otherwise is giving very promising results.
In this work, we present a novel method combining well-established techniques for material processing with low-cost and fast fabrication steps such as plasma deposition and etching techniques, using Nanosphere masking of polymeric materials. Several type of nanostructures (ranging from 70 to 250 nm) such as nano-domes and nano-wells consisting of materials with chemical and/or biological functionalities suitable for biosensor applications are produced in our laboratory. In this work some examples of this research activity will be illustrated. In particular the fabrication process and characterization of Poly Acrylic Acid nanodomes and of gold nano-wells will be described and discussed. The selective biological response the nano-patterned surface is demonstrated with protein assays i.e. BSA (Bovine Serum Albumin) is selectively bound to the functionalized nanostructures, whereas no protein adhesion is detected in the surrounding anti fouling matrix.

2 MATERIALS AND METHODS

PAA films have been deposited by PE-CVD in a capacitively coupled plasma reactor, previously described in ref. [1]. Gold film was deposited by classical magnetron sputtering. SiOx films were deposited by PECVD from HMDSO precursor (Sigma Aldrich) and oxygen etching was carried out in high density plasma source previously described in ref. [2].
PS (polystyrene) colloidal particles monodispersed in salt solution (Aldrich, Average Diameter: 500 ± 50 nm Concentration: 2% of solid content) were deposited onto the polymeric substrates by spin coating. A small drop (1 μl) of nano-particles solution (thermalized at the room temperature) was casted on the substrate with the spin-coater off and then accelerated (Average acceleration was varied between 1000 and 2000 $rp(m)^2$) to the final velocity varying between 500 and 5000 rpm). The spin-coater was stopped after the solvent evaporation.
BSA, as received from the supplier (Aldrich) was dissolved in PBS buffer solution (Fluka, pH = 7.4) with a concentration of 40 μg/ml. The samples were incubated in BSA solution for 30 min, washed in milli-Q water and then dried under a pure N_2 gas flow.

3. RESULTS AND DISCUSSION

3.1 Formation of colloidal mask

Nanosphere lithography is a reliable method to produce nano-topography over large area surfaces [3,4]. It uses the ability of the nano-sized particles to organize themselves on a surface to form some nano-masks. After plasma etching or deposition operation, nano-features can be transferred on the substrate. The nano-spheres adsorption on the surface

can be done by spin coating on surface treated by surfactant [3] or electrostatic adsorption using the electric charge contrast between particles and the substrate [4]. In both methods, the particles adsorption is controlled by the physico- chemical properties of the surfaces.
These two methods present some drawbacks for our application since both use chemical products, which can modify the chemical properties of the functionalized surface. In this paper, we use the PAA films hydrophilic character for the deposition of the nanospheres on the surface to avoid the use of a surfactant.

3.2 Polymeric nano-domes

The aim of this paragraph is to describe a method to create functional nano-domes over an anti-fouling matrix. The process is described in figure 1. The colloidal mask was created on the PAA layer deposited on a PEG layer. The oxygen plasma etches the PS and the unmasked PAA between the particles. An accurate control of the plasma etching time was necessary to avoid the over etching of the PAA. Indeed, the PAA over etching is harmful since the oxygen plasma strongly reduces the concentration of the functional PAA group on the surface. The etching time to avoid acrylic acid etching was 90 seconds. Some residual PS beads remained on the top of the sculptured PAA nano-domes. These residual beads are rinsed away by an ultrasonic bath in ultra-pure water. A SEM picture of the resulting nano-structured surface is shown in figure 2.

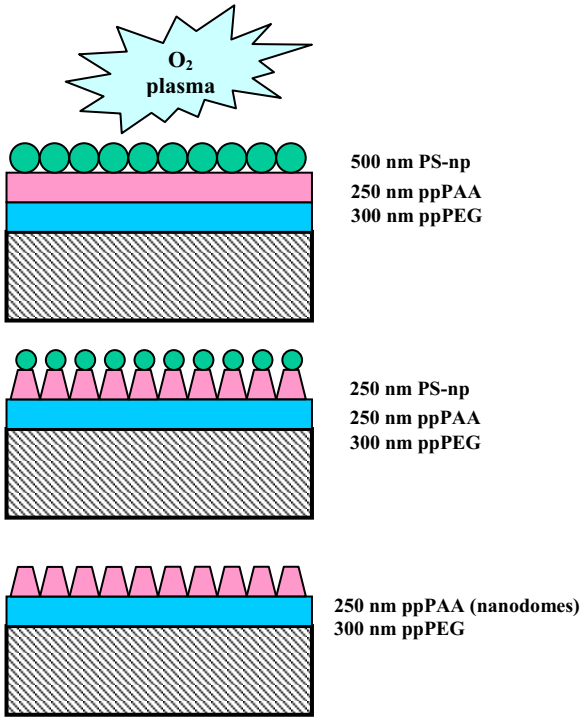

Figure 1 : Method of Polymeric nano-domes creation

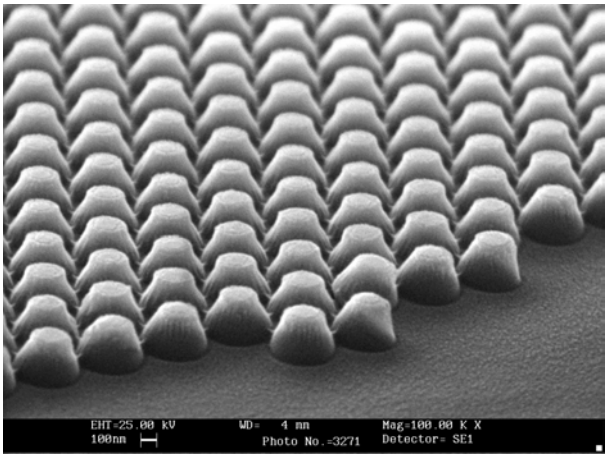

Figure 2: PAA nanodomes over antifouling matrix

The resulting surface presents 2-D crystalline PAA nano-domes (diameter: 250 nm) on a anti fouling surface..

3.3 Metallic nano-wells

In this case the aim is to create a contrast between a metal (e.g. Gold) and a silica surface in order to induce the selective absorption of Self Assembled Monolayers (SAM) with contrasted functionalities.
The process is described in figure 3. A thin (30 nm) PAA buffer layer is deposited on the gold surface in order to induce the formation of the PS nanomasks (Figure 3a). The PS beads are then etched by oxygen plasma as well as the unmasked PAA (Figure 3b). The plasma process is stopped before the complete removal of the particles. By this method it is also possible to control the well size and average distance. After the mask modification by oxygen plasma, the SiOx layer is deposited by PECVD (Figure 3c). The residual PS beads and PAA are then removed by an oxygen plasma cleaning process which does not affect the SiOx and gold surfaces (Figure 3d). The result (figure 4) is a nano-patterned surface with gold nano-wells over a SiOx matrix. The dimensions of the patterned can be modulated by changing the PS size and the plasma process parameters (etching and deposition times). These gold nano-wells are promising candidate for nano-electrodes to be used in electrochemical based sensors.

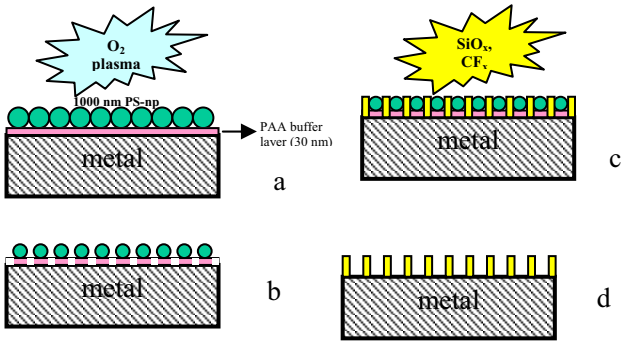

Figure 3: Method of nano-well creation.

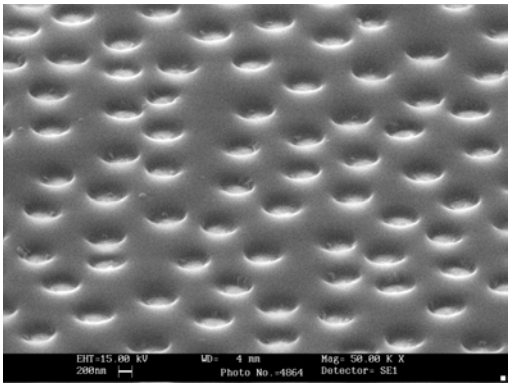

Figure 4 : Gold nano- well on SiOx matrix (diameter 250 nm)

3.4 Protein interaction assays

The first assays were done with proteins (BSA). Figure 5 shows that spherical BSA clusters are preferentially bounded to the functional plateau... The results outlined in this work are very promising for the biochip application; a great opportunity for these applications is the possibility to control the surface distribution of the nano-structures in a macroscopic area of the devices

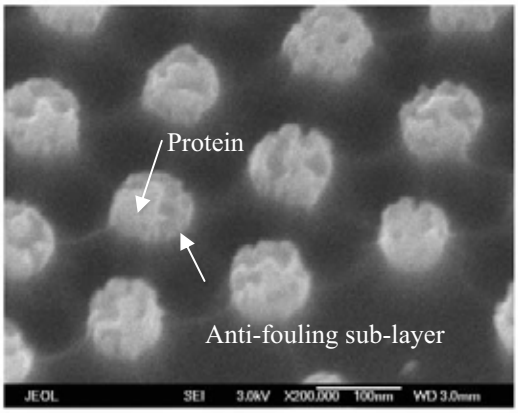

Figure 5: BSA protein selectively bounded on domes top surface.

REFERENCES

[1] Rossini N.; Colpo P.; Ceccone G.; Kandt K.D.; Rossi F., *Mater. Sci. and Eng. C* **2003,** *23*, 353-358
[2] Meziani T. ; Colpo P. ; Rossi F., *Plasma Sources Sci. Technol.* **2001,** *10*, 276–283
[3]. Hulteen J. C. ; Van Duyne R P., *J. Vac. Sci. Technol. A* **1995,** *13(3)*, 1553-1558
[4] Hanarp P.; Sutherland D. S.; Gold J.; Kasemo B., *Colloids and Surfaces A: Physicochem. Eng. Aspects* **2003,** *214,* 23-36