Application of plasma processes in NanoBiotechnology

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Abstract. We present an overview of the possibilities offered by the combination of plasma polymers deposition and nanopatterning by colloidal and electron beam lithography. It is shown that chemical and topographical patterns can be obtained on different substrates, with dimensions of the order of a few 100 nm. Two examples of applications of these nanostructures for protein adsorption studies are presented.

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1 Introduction

The fabrication of patterned surfaces at the nanoscale with a well defined geometry and a controlled chemistry is a fundamental step in the field of NanoBiotechnology [1–3]. Patterned surfaces with contrasted physico-chemical functionalities e.g. protein adhesive/non-adhesive, hydrophilic/hydrophobic, acid/base provide unique tools in a large field of applications such as tissue engineering [4,5], cell behavior investigations [6–8], artificial growth of neurons networks [7], or microfluidics, where the nanostructure lead to very unique effects related either to the kinetics of biochemical reactions at the interface [9,10], or launching of biological responses from living cells [5]. Numerous methods have been developed for the production of such surfaces and are based essentially on the use of Self Assembled Monolayers (SAM) [11,12] together with microcontact printing [13,14] or electrodeposition of functional polymers [15]. These techniques present several limitations either linked e.g. to the substrate that has to be used — Gold or Silicon in the case of SAM, or, in the case of microcontact printing, to the resolution that can be obtained [16]. We present here a summary of the work we did, based on the use of plasma assisted deposition of functional polymers and their local transformation and patterning by a combination of techniques, namely colloidal and electron beam lithography.

The advantage of using plasma polymers resides in the fact that they can be deposited on virtually any substrate and at low temperatures. The processes used allow, by the choice of the right precursors, to deposit materials a very large contrast of properties and chemical functionalities, such as –COOH [17], -NH$_2$ [18,19], or anti fouling polymers based for instance on polyethylene oxide (PEO) [20].

Due to their high degree of cross-linking, these coatings are resistant to classical polar solvents and can be used in standard lift-off processes [21].

The plasma polymers can be then assembled in structures with nanometric dimensions by combination of colloidal lithography, depositions and etching, or as well as by direct modifications by electron beam lithography. We present below a summary of these achievements and an illustration of the properties of the bio/non bio interfaces that can be obtained.

2 Experimental details

In this work, two different type of plasma polymers are used, namely plasma polymer of acrylic acid (PAA) containing COOH moieties and a protein and cell repellent plasma polymerized Polyethylene Oxide (PEO)-like surface. The choice of the acrylic acid is motivated by the fact that the carboxylic functions are able to promote a large amount of chemical reactions with different biomolecules (enzymes, antibodies, DNA...).

2.1 Substrate preparation

Double side polished silicon wafers (Si (100), diameter 50 mm; resistivity 1–20 Ω cm) supplied by ITME (Warsaw, Poland) have been used. They were cut in 1 × 1 cm squares and were cleaned before utilization by sequential sonication (5 min/cycle) in trichloroethylene, acetone and ethanol.

2.2 Films processing

The deposition process of the plasma polymerized PEO-like and PAA films are described in details
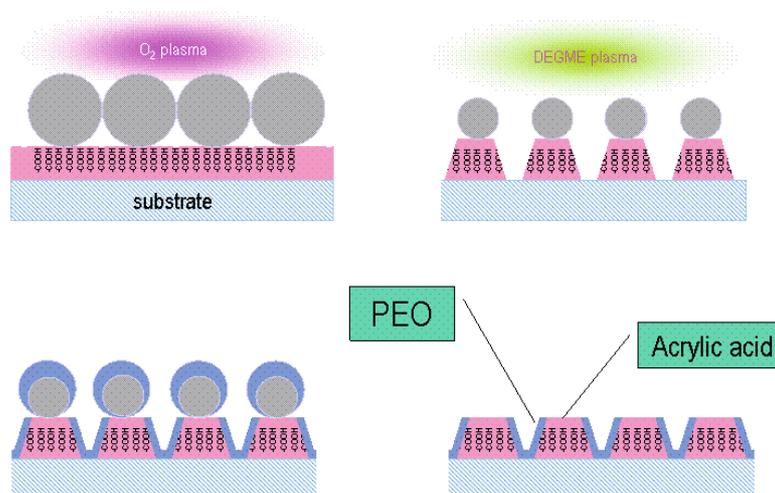
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Fig. 1. (Color online) Operations used for colloidal lithography patterning a a polymeric surface. (a) PS beads are deposited on a PAA films on a Si substrate and etched by an O_2 plasma. One then obtain the structure as represented in (b). A PEO layer is then deposited on the structure and covers the complete surface (c). After sonication, the beads are removed and one obtains the structure presented in (c) where nanodomains of PAA are distributed in a non adhesive matrix of PEO (d).

elsewhere [17,18,21]. Briefly, the systems used were capacitively coupled reactors with two symmetrical parallel-plate electrodes. For PEO-like coating, plasma polymerization was carried out by using a radio frequency generator (13.56 MHz) in pulsed mode (time on = 10 ms, time off = 100 ms, nominal power = 5 watts) of pure Diethylene Glycol Dimethyl Ether (Di-glyme), $(CH_3OCH_2CH_2)_2O)$ vapors (Sigma Aldrich, used as received). For PAA, acrylic acid vapours were mixed with Ar in pulsed RF mode (13.56 MHz; pulsed plasma: time on = 4 ms; time off = 36 ms). The films obtained are uniform and their thickness can be adjusted between 20 and 200 nm.

3 Results and discussions

3.1 Surface patterning using colloidal lithography

The objective of the patterning of a surface by colloidal lithography is to create 2-D crystalline structure of polystyrene (PS) beads on the PAA, to be used as masks during subsequent etching and deposition operation. The mask self assembly is performed by evaporation of solvent containing the PS beads in suspension. During the de-wetting and evaporation, the colloidal particles can be arranged in different geometrical configurations in order to minimize the space and the free energy of the system [21,22].

First, the PAA layer (thickness = 200 nm) was deposited on a Si substrate as described above. Then, a micro-drop (Drop Volume = 5 μl) of PS beads (typically of 200 to 500 nm diameter) suspension is deposited on the 1×1 cm^2 samples surface. The bulk volume of the drop is removed by a micro-pipette, in order to obtain a very thin layer of PS beads suspension on the surface. The sample is then spun to produce a slow evaporation of the liquid. It is thus possible to create macroscopic homogeneous areas covered by mono-layered nano-beads of few mm^2 with a surface coverage ranging from 70% to 100%. The nano-mask pattern is then transferred to the PAA layer by O_2 plasma etching (Fig. 1a). The etching operation has to be accurately controlled in order to create the nanostructures on the PAA layer and avoid over-etching. Once the polymeric nano-structures are created on the surface, the chemical contrast is formed by the plasma deposition of the PEO-like coating. The anti-fouling layer is deposited on the surface through the nano-masks (Fig. 1c). The deposition is isotropic and the regions unprotected by the masks (both the partially and the totally uncovered regions) are coated. The residual nano-sphere mask is then lifted-off by an ultrasonic treatment of the surfaces in ultra-pure water. The final surface is composed of areas of carboxylic functionalities of a few hundreds of nm surrounded by the anti-fouling layer (Fig. 1d). The AFM image of Figure 2 shows the typical 'nanodome' structure, the dimensions of which depend on the initial size of the beads, here 500 nm, the PAA layer thickness and the etching time, typically a few hundreds of nm in diameter and few tenth of nm in height.

3.2 Direct surface patterning using electron-beam lithography

However, colloidal lithography is limited in the geometry of the patterns that can be produced (shape of the patterns, pitch, size, and distribution). To overcome these limitations, electron-beam lithography is an attractive technique which can offer a high resolution definition (below 10 nm using appropriate sequence of developing [22,23] and the possibility to pattern large areas with virtually any motive [24].
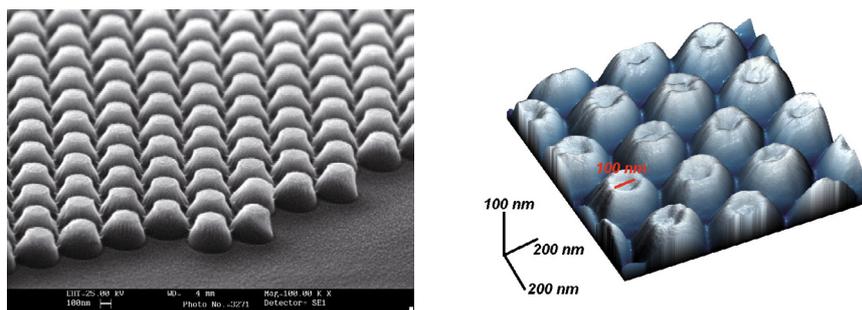
Fig. 2. SEM and AFM pictures of the structures obtained by colloidal lithography.
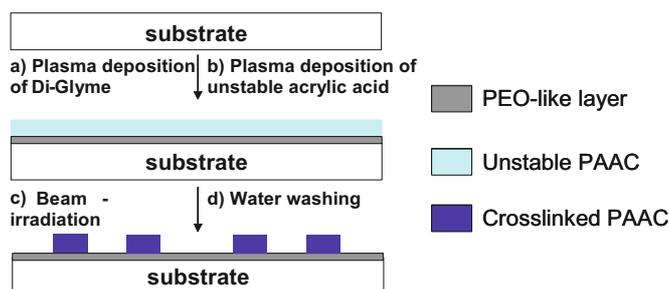

Fig. 3. (Color online) Electron-Beam lithography on plasma polymers: A PEO layer is first deposited on a Si substrate (a). A second layer of unstable PAA is deposited on the PEO (b). e-beam irradiation stabilizes locally the PAA (c). After washing, one obtains a nanostructure of stable PAA domains on a non adhesive PEO background (d).

The process of fabrication is described in Figure 3. First, a 20nm layer of polyethylene glycol is plasma deposited on a Si substrate in order to form the non-adhesive matrix [25]. After plasma deposition of the PEO-like layer, a second water soluble layer is deposited by plasma polymerization of acrylic acid in the monomer rich region. The conditions of deposition of this layer are similar to the one of PAA previously described but with a lower power of deposition (nominal power of 10 watts, operating in pulsed mode with a t_{on} of 4 ms and a duty cycle of 10%). After deposition of the water soluble layer, the surface is exposed to the electron-beam with an energy of 20 keV and incident dose of 1600 $\mu C/cm^2$, then immerged for 5 min in water and dried under nitrogen flow. The chemistry of the stabilized PAA layer has been determined by μ spot XPS on a micro patterned surface (100 × 100 μm^2). The XPS reveals that the chemistry of the stabilized layer is modified as compared to the initial film, because of the cross linking occurring during irradiation. The chemistry of the film thus produced is similar to plasma polymerized acrylic acid layer produced at optimum plasma density and used for bio specific applications [26] with a percentage of retention of COOH groups around 7–10%. More experimental details can be found in [29].

Figure 4 shows AFM and SEM images of functional nano-structures with different heights and shapes created with the patterning process described in this study. Well defined features sizes down to 200 nm in lateral resolu-

tion can be fabricated with several heights (from 80 nm to 500 nm). It must be noted that the resolution limit obtained (or the order of 100 nm) depends on the e-beam energy and can be much improved by working at lower energies (typically down to 5 keV). These images proves the feasibility of combining electron-beam lithography and plasma processes for direct patterning of surfaces with well defined nano-scale dimensions.

3.3 Protein adsorption experiments

The nanostructures produced by colloidal and electron-beam lithography have been extensively studied with different biological tests and protein adsorption experiments [10,27–30]. We present below two significant examples indicating the particular behaviour of nanostructured as compared to homogenous surfaces.

In order to illustrate that the potential of these surfaces as biosensors platforms, nanostructured micro-patch surface produce by e-beam stabilization of acrylic acid on a PEO background has been exposed to a fluorescent Poly-L-Lysine (PLL) solution. The fluorescent contrast observed in Figure 5 after the incubation shows that PLL is adsorbed selectively on the PAA patches and that the PEO-like has kept its anti adhesive properties.

In a second experiment, an Enzyme Linked Immunosorbent Assay (ELISA) test was performed on the surfaces produced by colloidal lithography. The protocol is described briefly here: first, a monoclonal antibody (Anti-Ovabulmin 50 μg/ml overnight) is immobilized on the surface. Bovine Serum Albumin (BSA) (200 μg/ml) is used in this case as a blocking agent for nonspecific adsorption. Then the sample is exposed to the antigen labeled with Horseradish Peroxidase (HRP) (Ovalbumin-c-HRP, 10 μg/ml). Then tetramethylbenzidine (TMB) is added to the solution. TMB forms a blue product when allowed to react with HRP. The reaction is halted by addition of HCl and the solution turns yellow. The intensity of the colour of the solution is measured by dispersive spectrophotometer as the absorbance at the wavelength of 450 nm. The measured absorbance at 450 nm, normalized by the active area for the different surfaces tested is shown in Figure 6. The results show that the normalized absorbance of the nanopatterned surface is about 4 times higher than the one obtained with the non-structured surface (PAA).
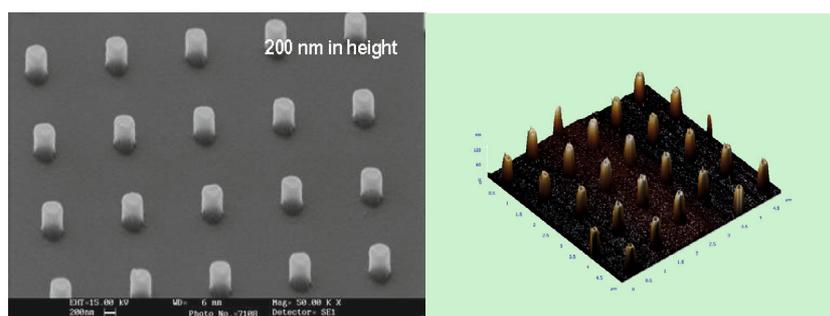
Fig. 4. (Color online) AFM and SEM pictures of nanostructures obtained by e-beam patterning of a unstable PAA film. Typical dimensions in height can vary from 100 to 500 nm.
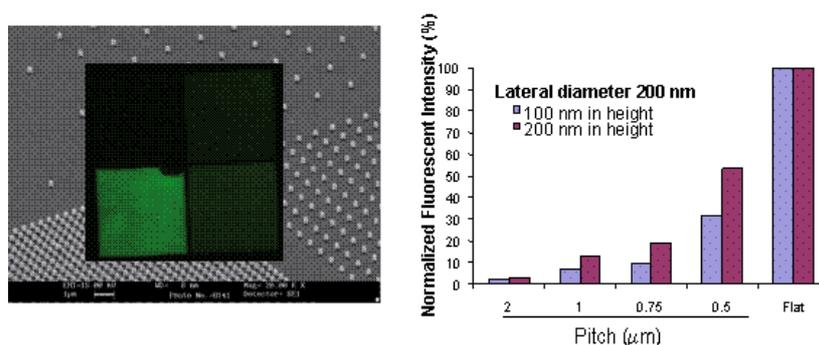

Fig. 5. (Color online) (a): SEM and fluorescent (include inside the SEM image) of Nanopillars ($h = 100$ nm, $d = 200$ nm) of stabilized PAA on PEO-like surface and corresponding relative fluorescent intensity after incubation with PLL for different height and pitch.
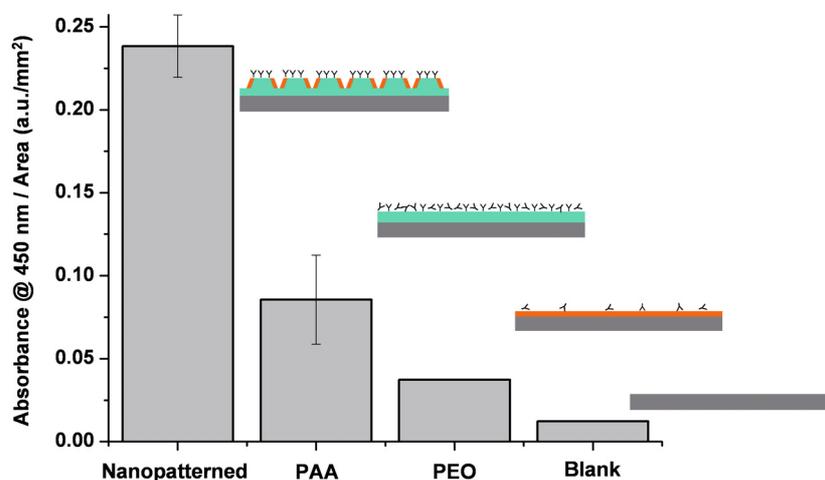

Fig. 6. (Color online) Results of the ELISA test done on 3 different surfaces: Plasma deposited PEO and PAA homogenous surfaces. Nanostructured surfaces, dome size 100 nm, pitch 500 nm. The results show a clear enhancement of the response due to the nanostructures.

This means that a much higher number of antibody/antigen reactions took place on the nanostructured surface as compared to the other tested surfaces. The explanation of this phenomenon of amplification is found with different detection techniques and experimental procedures and is still under investigation. The level of amplification depends on the size of the nanostructure and increases as the size decreases. A possible explanation is linked to a better orientation of the antibodies on a nanostructured surfaces and a reduction of the steric hindrance.

4 Conclusions

In this study we have demonstrated that colloidal and electron-beam lithography can be combined with plasma

processes for production of high quality micrometric and sub-micrometric (down to 200 nm) patterned surfaces with arbitrary geometries and controlled chemistry. Moreover, due to the versatility of the plasma processes, this technique can be easily extended to a large panel of surface chemistries and substrates. We find that these structures offer very interesting responses in cell cultures in as well as bioanalytics, with an increase of sensitivity of the immunoreaction tested. This increase, which depends on the size of the nanostructures, is particularly interesting in the case of low concentration of analyte or in the case of expensive reagents. It can also be applied for high density microchips for high throughput testing.

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