Role of plasma surface engineering in NanoBiotechnologies"

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Extended Abstract

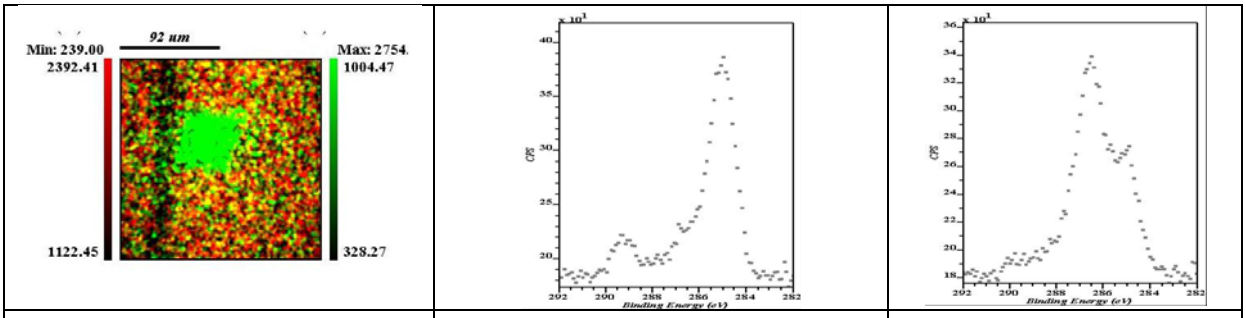
One of the major challenges for the development of analytical devices for biological analysis relies on the ability to design advanced surfaces with controlled interaction with the biological world[1]. Surface functionalization techniques provide those bio-interfaces: appropriate surface physico-chemical properties are able to control the conformation and activity of the immobilized biomolecules. The subsequent technological step is the combination of different bio-functions in micro- and nano-patterns on the surfaces. For instance structuring the surface in adhesive and non adhesive zone in order to preferentially guide the cell growth is one of the most interesting tools for the development of cell chips and for tissue engineering[2-3].The requirement of further integration scales and the study of the special behaviour of the biomolecules interacting with nanostructured materials have been the two main motivations for the development of submicron patterning techniques[4]. For instance an increase of several order of magnitude of analysis capacity in biosensing devices together with lower detection limits are envisaged[4-5].

Plasma assisted techniques are interesting alternatives to produce functionalized surfaces with controlled micro- and nano-patterns: they provide high level functionality surfaces with good stability on different substrates and are compatible with different micro- and nano-patterning techniques [6-7]. For examples, surfaces with different functional groups such as ammine[8-9], carboxy(COOH)[10-11], hydroxy[12-13], and aldehyde[14-15] have been deposited with different degree of success in different laboratories with different precursors and deposition conditions. These kinds of functionalized surfaces are routinely used for coupling biomolecules such as proteins, [16-17-18] DNA[19-20], polysaccharides[21-22], enzymes[23], and pectins[24], but to guide and control cell-surface interactions. [25-26-27] Furthermore, plasma treatments have been successfully used to deliver cell therapy for wound healing,[28] and for decontamination of surfaces[29-30]

In this work we will report some examples of micro- and nano-functional surfaces provided by plasma processes in combination with Optical Lithography, Electron Beam Lithography and Colloidal lithography for applications in different biosensing devices. The different experimental set-ups and reactor geometries used in these studies are described in detail in refs. [2-3_31-32-33]

Micropatterned surfaces were produced by a spatial arrangement of different functional domains by a combination of plasma polymerisation and photolithography: non-fouling patterns were made of (PEO)-like polymers obtained by pulsed plasma polymerization of diethylene glycol dimethyl ether while fouling surfaces were composed of PEO coatings with a low concentration of ethylene oxide groups and films containing amino groups (from allylamine monomer) or carboxylic groups (from acrylic acid monomer) obtained by plasma polymerization. The optimization of the deposition processes of plasma polymerized acrylic acid (ppAA) and poly-(ethylene oxide) PEO-like films have been investigated by means of different analysis techniques, namely XPS, ToF-SIMS, AFM both for flat and patterned surfaces. For example, in Figure 1 typical XPS photoemission image of a micropatterned

ppAA/PEO-like surface is illustrated together with the C1s core level spectra recorded in the ppAA region and in the PEO-like surrounding matrix. As can be seen the carboxyl retention of the ppAA (peak at ~290.2eV) is about 10% whilst the presence of the peak at about 286.5eV confirms the PEO-Like character of the surrounding matrix.



(a) **(b)** **(c)**

Figure 1: (a) XPS C1s image of a ppAA/PEO-like micropatterned surface, (b, c) C1s core level spectrum (27μm spot) of the ppAA (green) and PEO-like region (red)

Cell adhesion studies (with L929 mouse fibroblasts) on patterned surfaces showed that the fibroblasts only adhere on the patterns, whereas the background stays uncovered (Figure 2)[3].

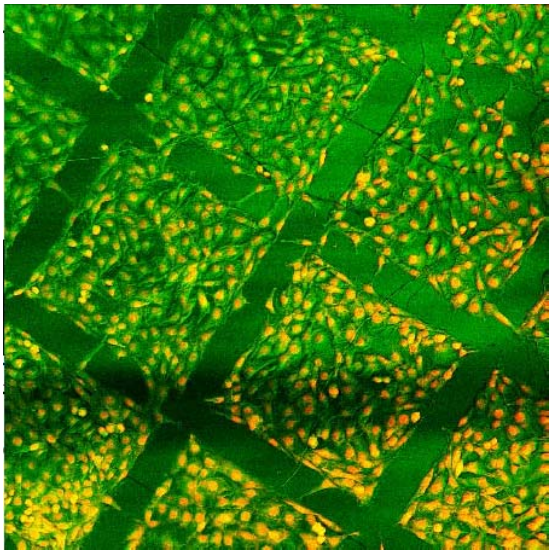


Figure 2. Optical microscope picture of the fibroblasts growing selectively in the fouling areas on a fouling-antifouling surface micropatterned by optical lithography. (Field of View: 500μmx500μm)

On the other hand, nano-patterns of fouling-antifouling areas have been realized by combining Electron-beam Lithography and Colloidal Lithography techniques with plasma processes: in particular carboxylic functionalized nano-domes in a PEO-like anti-fouling matrix have been produced [4-31]. In figure 3 (a) an AFM image of the surface obtained is illustrated. As can be seen, the structures have a conical shape with a diameter of about 150nm and a 500nm distance. The selective adhesion of different proteins (BSA, Avalbumine) and antibodies were also proved as illustrated in Figure 3(b), where an SEM image of the nanostructured surface after immersion in protein solution is presented. As can be seen, biomolecules are selectively immobilized onto carboxylic functional nano-domains, leaving the anti-fouling matrix clear. Moreover Enzyme-Linked Immunoadsorbent Assay

experiments were set-up showing that nano-patterned surface constrains the immobilization of the antibodies in a biological reactive configuration, thus significantly improving the device performances as compared to more conventional non-patterned surfaces.

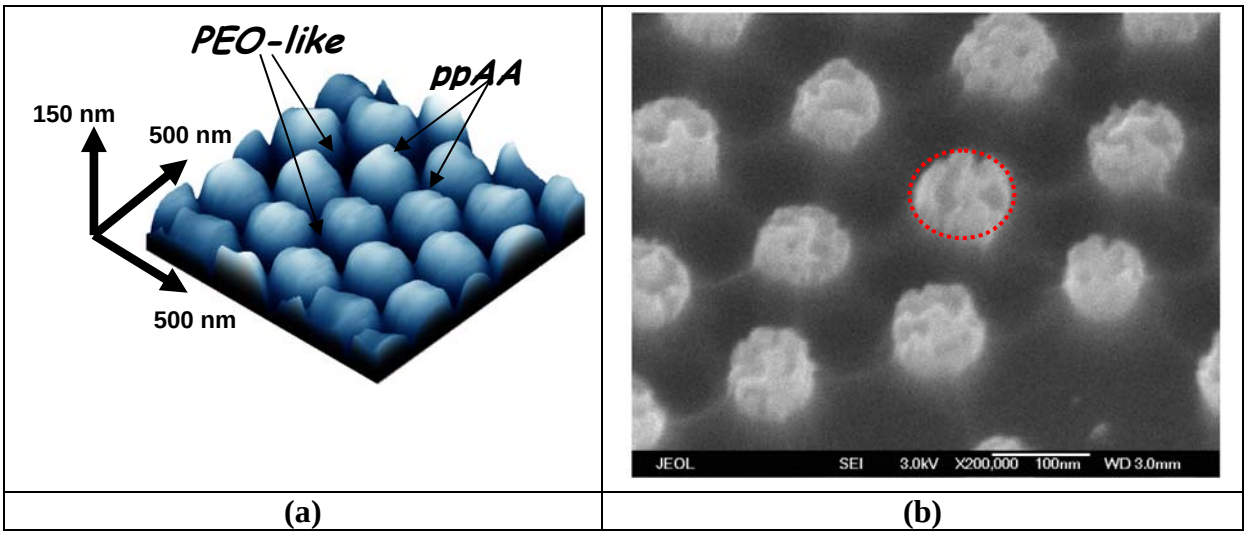


Figure 2. (a) AFM picture of a ppAA/PEO-like nanostructured surface. (b)SEM picture of protein (BSA) selectively absorbed on the ppAA/PEO-like nano-structures. The center of each dome is COOH-functionalized surrounded by a PEO-like anti-fouling matrix with a hexagonal arrangement: the bright objects in the picture (one indicated with a red circle) are the proteins.

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References

[1] B. G. Keselowsky, D. M. Collard, and J. Garcia, *PNAS* **2005,** *102*, 5953-5957
[2] F. Bretagnol, L. Ceriotti, M. Lejeune, A. Papadopoulou-Bouraoui, M. Hasiwa, D. Gilliland, G. Ceccone, P. Colpo, and F. Rossi, *Plasma Processes and Polymers* **2006,** *3*, 30-38
[3] F. Bretagnol, A. Valsesia, G. Ceccone, P. Colpo, D. Gilliland, L. Ceriotti, M. Hasiwa, and F. Rossi, *Plasma Processes and Polymers* **2006,** *3*, 443-455
[4] A. Valsesia, P. Colpo, P. Lisboa, M. Lejeune, T. Meziani, and F. Rossi, *Langmuir* **2006,** *22*, 1763-1767
[5] H. Agheli, J. Malmstroem, E. M. Larsson, M. Textor, and D. S. Sutherland, *Nano Letters* **2006,** *6*, 1165-1171.
[6] K.S. Siow, L. Britcher, S. Kumar, H.J. Griesser, *Plasma Proc. Polym.* **2006,** 3, 392
[7] R. d'Agostini, P. Favia F. Fracassi (eds.) *Plasma Processing of polymers*, **1997**, NATO ASI series vol. 346
[8] P. K. Chu, J. Chen, L.P. Wang, N. Huang, *Mat. Sci. Eng.,* **2002**, 36, 143
[9] W.R. Gombotz, A.S. Hoffmann, *J. Appl. Polym. Sci,* **1998,** 42, 285
[10] R. Daw, S. Candan, A.J. Beck et al., *Biomat.* **1998,** 9, 1717
[11] P. Rossini, P. Colpo, G. Ceccone, K.D. Jandt, F. Rossi, *Mat. Sci. Engin. C,* **2003,** 23, 353
[12] H.J. Griesser, R.C. Chatelier, *J. Appl. Polym. Sci*, **1990,** 46, 361
[13] S. Ertel, B.D. Ratner, T.A. Horbett, *J. Biomed. Mat.,* **1990,** 24, 1637
[14] H. Thissen, K. McLean, G. Johnson et al., *Trans. Am. Soc. Biomat.* **1999**, 25, 450
[15] M. Leich, N.M. Mackie, K.L. Williams, E.R. Fisher, *Macromol.,***1998**, 31, 7618
[16] Y. Ito, M. Kajiara. Y. Imanishi, *J. Biomed. Res.,* **1991**, 25, 1325
[17] S. Sano, K. Kato, Y. Ikada, *Biomat.,* **1993,** 14, 817
[18] B. Gupta, C. Plummer, I. Bisson, et al., *Biomat.,* **2002**, 23 863
[19] Z. Zhang, W. Knoll, R. Foerch, *Surf. Coat. Technol.,* **2005,** , 200, 993
[20] L. Dai, A.W. StJohn, J. Bi et al.,*Surf. Int. Anal., **2000,** 29, 46*
[21] K. M. McLean, G. Johnson, R.C. Chatelier, et al., *Coll. Surf. B*, **2000,** 18, 221
[22] F. Garbassi, M. Morra, E. Occhiello,(eds.), *Polymer Surfaces: from Physics to Technology*, **2002,** Wiley&Sons, USA
[23] I. Gancarz, J. Bryjak, G. Pozniak, W. tylus, *Eur. Polym. J.,* **2003,** 39, 2217
[24] M. Morra, C. Cassinelli, M.D. Nagel, G. Ceccone , et al, *Biomacromol.,* **2004,** 5, 2094
[25] E. Sardella, P. Favia, R. Gristina, et al., *Plasma Proc. Polym*, **2006,** 3, 456
[26] R.M. France, R.D. Short, R.A. Dawson, etal., *J. Mat. Chem.,* B1998, **8, 37**
[27] B.D. Ratner, S. J. Bryant, *Ann. Rev. Biomed. Eng.,* **2004,** 6, 41
[28] D.B. Haddow, S. MacNeil, R.D. Short, *Plasma Process. Polym.,* **2006,** 3, 419
[29] F. Rossi, O. Kylian, M. Hasiwa, *Plasma Process Polym.,* **2006,** 3, 431
[30] M. Moisan, J. barbeau, S, Moreau, et al., *Int. J. Pharm.,* **2001,** 226, 1
[31] A. Valsesia, P. Colpo, M. M. Silvan, T. Meziani, G. Ceccone, and F. Rossi, *Nano Letters* **2004,** *4*, 1047-1050
[32] A. Ruiz, L. Ceriotti, L. Buzanska, et al., *Microel. Engin.***2007,** 84, 1733
[33] F. Bretagnol, O. Kylian, M. Hasiwa, et al., *Sensors and Actuators B,* **2007**, B123, 283