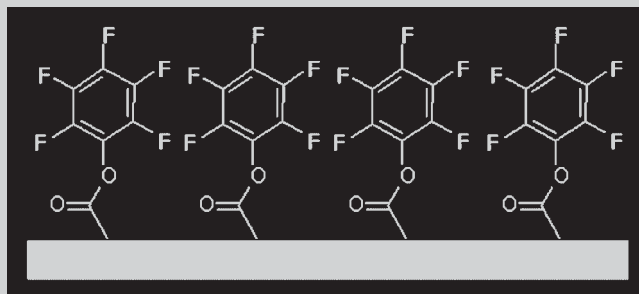


Summary: Material coating of surfaces can enhance receptivity for cells and biological compounds. Existing plasma coating technologies and possible materials are limited. A new polymer from pentafluorophenyl methacrylate (PFM) monomer was synthesized, and was plasma enhanced chemical vapor deposited on silicon wafers. The optimal plasma polymerization parameters for the PFM monomer and its copolymerization with the cross-linking agents 1,7-octadiene and 1,4-butanediol divinyl ether co-monomers were established. All the resulting polymer coatings leave the labile pentafluorophenyl group on the surface, enabling a rapid reaction with an amino-terminated biotin ligand and allowing

layer-by-layer self-assembly of biotin-streptavidin. In addition, the deposited polymer layers showed an extremely flat morphology with a nanoscale average roughness. This approach provides an easy means of obtaining functionalized surfaces which can enhance and control the biocompatibility of bulk materials. Merging the versatility of plasma polymerization processes, via simple monomers and reaction conditions, with biological platforms that enable target of cell adhesion brings us closer to the ultimate goal of controlling cell function through structured surfaces for their application in tissue engineering.



Schematic representation of a functionalized surface obtained by plasma polymerization of the PFM monomer, showing labile pentafluorophenyl groups on the surface.

Fabrication of Bioactive Surfaces by Plasma Polymerization Techniques Using a Novel Acrylate-Derived Monomer

Laia Francesch,¹ Elena Garreta,¹ Mercedes Balcells,^{1,2} Elazer R. Edelman,^{2,3} Salvador Borrós*¹

¹Laboratori de Ciència dels Materials, Barcelona Bioengineering Center, Institut Químic de Sarrià-Universitat Ramon Llull, Via Augusta 390, Barcelona 08017, Spain

Fax: +34-932-056-266; E-mail: s.borros@iqs.url.edu

²Harvard University-Massachusetts Institute of Technology, Division of Health Sciences and Technology, Cambridge, MA 02139, USA

³Cardiovascular Division, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

Received: April 27, 2005; Revised: August 6, 2005; Accepted: August 22, 2005; DOI: 10.1002/ppap.200500042

Keywords: biomaterials; biotin-streptavidin; immobilization of molecules; pentafluorophenyl methacrylate; plasma polymerization

Introduction

Targeting materials to specific cells,^[1] designing materials that can sense biochemical signals^[2] and developing materials with improved biocompatibility remain challenges in biomaterials development.^[3] Significant effort remains

in increasing the cell recognition and attractiveness of biomaterial surfaces. Modified materials stimulate cell adhesion, growth and proliferation,^[4] while maintaining the mechanical attributes of the bulk material.^[5–7] Cell attachment to bioactive surfaces may be enhanced through linkage of biological ligands specific for cell-substrate

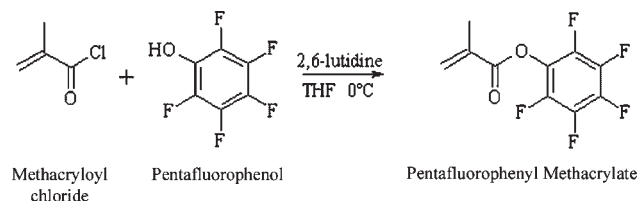
interactions.^[8,9] One broadly used approach to immobilizing ligand motifs on biomaterial surfaces consists of creating covalent attachments to reactive polymer side chains.^[10] To this goal, plasma polymerization allows deposition of thin films onto substrates of complex geometry with a uniform distribution of functional groups.^[11,12] The integrity of the functional groups, however, remains a challenge in any plasma polymerization process. Additionally, the adhesion between the film obtained and the substrate needs to be taken into account.^[13]

Biotin-streptavidin technology, a valuable tool in modern biotechnology, is used for immobilization of molecules to solid phases,^[14] signal amplification in bioaffinity assays,^[15,16] and targeted drug delivery.^[17,18] In this context, microcontact printing (μ -CP) offers relatively simple, easy to implement, and attractive methods for micropatterning of such biomolecules.^[19] The target surfaces must however be extremely reactive for a successful covalent linkage of biomolecules. In other words, the presentation of labile groups, such as voluminous active esters, is a requisite for the substitution of such groups by the biomolecule of interest.^[20–23] Classically plasma vapor deposition utilizes derivatives of parylene, and yet the synthesis processes involved are complex and lengthy.^[24] This study sought to develop a feasible alternative to complex functionalized monomers. A new acrylate-type monomer, pentafluorophenyl methacrylate (PFM), was synthesized and used for plasma polymerization. Although methacrylate derivatives of pentafluorophenol are well known,^[25] their use as monomers for plasma polymerization has not been studied in depth. We established the different parameters needed to maintain the monomer functionality under pulsed radio-frequency (RF) plasma polymerization. Plasma polymerized films presented the labile pentafluorophenyl group on the surface, which was optimum for subsequent biotin binding.

Experimental Part

Materials

Florosil[®] (60–100 mesh), methacryloyl chloride (+98%), 2,6-dimethylpyridine (2,6-lutidine, 97%), 2,3,4,5,6-pentafluorophenol (99%), 1,7-octadiene (98%), 1,4-butanediol divinyl



Scheme 1. Chemical synthesis of pentafluorophenyl methacrylate (PFM).

ether (98%) were obtained from Aldrich (Milwaukee, USA) and used as supplied. (+)-Biotinyl-3,6,9-trioxaundecanedi-amine, streptavidin, fluorescein-conjugated streptavidin and Tween 20 detergent were obtained from Pierce (IL, USA). Phosphate buffered saline (PBS, pH 7.4) and bovine serum albumin (BSA) were obtained from Gibco (New York, USA) and Fluka (Switzerland), respectively. Tetrahydrofuran (THF) was obtained from Aldrich (Milwaukee, USA).

PFM Synthesis

Methacryloyl chloride (8 ml, 82 mmol) was added drop-wise to a stirred solution of pentafluorophenol (7.6 g, 41 mmol) and 2,6-dimethylpyridine (6.6 g, 62 mmol) in THF (100 ml) at 0 °C (see Scheme 1). After 10 min stirring at the same temperature, the precipitate was filtrated and washed with THF (50 ml). After solvent removal under reduced pressure, the PFM residue was purified by chromatography on a Florosil[®] column, using pentane as eluent, and obtained as colorless oil (7.0 g, 85%). The monomer was stored at 4 °C until use.

¹H NMR and ¹³C NMR spectra of PFM were determined in CDCl₃ as solvent on a Gemini 300 Varian spectrometer.

¹H NMR (CDCl₃/TMS, 300 MHz, ppm): δ = 6.454 (1H, dq, H_b), 5.914 (1H, dq, H_a), 2.093 (3H, CH₃, dd).

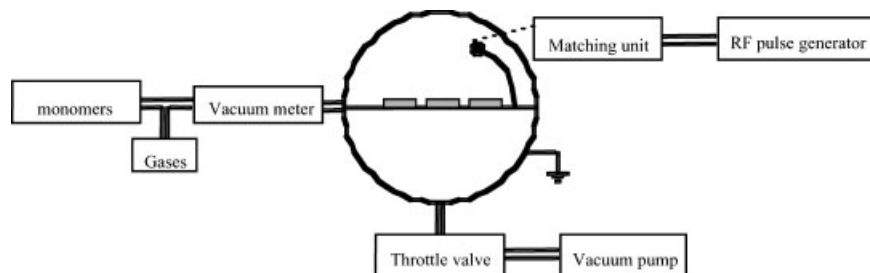
¹³C NMR (CDCl₃/TMS, 75 MHz, ppm): δ = 162.910 (1C, s, C=O), ca. 139.6 (5C, m, C–F), 133.626 (1C, s, H₂C=C), 129.786 (1C, s, H₂C=C), 18.272 (1C, s, C–CH₃).

IR spectra were obtained using a Nicolet Magna 5600 spectrophotometer.

IR (KBr, cm^{–1}): ν = 2950 (m), 1764 (s), 1625 (m), 1475 (m), 1100 (m).

Plasma Polymerization

The plasma deposition apparatus consisted of a stainless steel discharge vessel (diameter: 26 cm, length: 24 cm) parallel plate reactor (Scheme 2). The ground electrode was the reactor chamber, and the RF electrode was a stainless steel plate. The



Scheme 2. Scheme of the reactor.

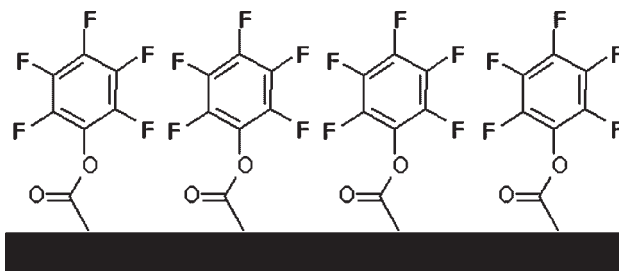
samples were located on this plate for polymerization. The RF electrode was connected to a RF pulse generator (13.56 MHz) through a matching network.

The gases or monomers were supplied via a standard manifold with gas fluxes adjusted with needle valves. The system pressure was determined using a Pirani type vacuum meter (MKS, USA), positioned between the reactor and a cold trap. The two stage mechanical pump (RV12 903, Edwards, GB), positioned after the cold trap, evacuated the vessel to a reactor chamber pressure of 0.1 mbar. All polymers were deposited on 100 oriented silicon wafers. All polymerizations were performed under pulsed plasma. The pulsed RF power setting was 10 W, with a duty cycle of 50% with a frequency of 15 Hz. Deposition times ranged from 2 to 8 h. PFM was used as synthesized. The monomer was preheated to reach the desired polymerization pressure. The monomer flow rate was set so that the pressure within the reactor was 0.3 mbar. PFM was also copolymerized with 1,7-octadiene (liquid, boiling point: 115.5 °C at 760 mmHg) and with 1,4-butanediol divinyl ether (liquid, boiling point 95 °C at 20 mmHg) as crosslinkers. Crosslinker/PFM ratios used were 3:1 and 6:1 for octadiene/PFM and 3:1 and 4:1 for divinyl ether/PFM.

All thin films obtained were characterized by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). XPS analysis was carried out using a Perkin Elmer PHI 5500 spectrometer (USA), equipped with a 350 W monochromatic AlK(alpha) X-rays source. Pass energy for the acquisition of C1s photoelectrons narrow scans was 23.5 eV. All XPS spectra were recorded at a take off angle of 45° relative to the surface normal under high vacuum conditions (the pressure in the main UHV chamber was maintained below 5×10^{-9} mmHg). Data analyses were carried out using Multipax software (PHI) using for calibration the C1s component of the unfunctionalized aliphatic hydrocarbon at a binding energy of 285.0 eV. AFM analysis was performed in tapping mode using a Nanoscope III Instrument (Digital Instruments, Santa Barbara, CA, USA). The roughness of the coatings was measured in terms of and root mean square roughness (R_q) and maximum roughness height (R_{max}). Film thickness was used as a measure of growth rate and determined after 2 h of deposition using a Dektak 6M profilometer (Veeco, USA).

Biotin and Streptavidin Linkage

Thin films previously obtained by polymerization of PFM (see Scheme 3), octadiene/PFM and divinyl ether/PFM were immersed for 2 h while shaking in a 10×10^{-3} M solution of (+)-biotinyl-3,6,9-trioxadecanedi-amine in distilled water. Subsequently, samples were washed for 30 min in buffer A consisting of PBS with 0.02% (v/v) Tween 20 and 0.1% (w/v) BSA. The detergent Tween 20 is a blocking agent composed of polyoxyethylene sorbitan monostearate that preferentially adsorbs to the background. It was used to wash away non-covalently bound biotin and to avoid non-specific adsorption of streptavidin.^[26] Then, samples were immersed for 1 h in a 10×10^{-3} M solution of fluorescein-conjugated streptavidin in buffer A while shaking. The surfaces were rinsed several times with PBS before examination done using an epifluorescence microscope (Nikon, New York). The streptavidin is labeled with a fluorescent die (in our case fluorescein 5'-isothiocyanate



Scheme 3. Schematic representation of a functionalized surface obtained by plasma polymerization of the PFM monomer, showing labile pentafluorophenyl groups on the surface.

(FITC)) which emits fluorescent light when excited at a certain wavelength. Therefore, if the streptavidin is bonded to the biotin, we can prove that by irradiating the sample at the characteristic wavelength of the fluorescent die. The emission of fluorescence by the sample indicates the presence of the biotin-streptavidin complex. In an epifluorescence microscope both the excitation and emission light travel through the objective. The key to the optics in an epifluorescence microscope is the separation of the illumination (excitation) light from the fluorescence emission emanating from the sample. In order to obtain either an image of the emission without excessive background illumination, or a measurement of the fluorescence emission without background “noise”, the optical elements used to separate these two light components must be very efficient.

Results and Discussion

Pentafluorophenyl Methacrylate Synthesis

The synthesis of the PFM followed a general synthesis pathway for acrylate derivatives with a yield of 85% which represents a 2.5 fold increase compared to yields previously reported using *p*-cyclophane derivatives.^[27] Sterically hindered pyridine was used as proton acceptor to limit the extent of an undesired side reaction: Michael addition of the pyridine to the acrylate. Moreover, an excess of the base was used to prevent the addition of hydrogen chloride to the activated double bond of the acrylate. The high basic pH of the 2,6-lutidine compared to pyridine increased the extent of deprotonation of the phenol.^[25] The reaction product was purified using a Florosil® chromatography column, resulting into a very pure form, as suggested by the NMR spectra. The IR spectrum of the pure PFM showed some characteristic bands (Figure 1a). Bands in accordance with alkane C–H stretching absorptions were present around 2950 cm^{-1} , while the band at 1764 cm^{-1} indicated a carbonyl stretching absorption of a carboxylate ester. Aromatic system stretching vibrations between 1625 and 1475 cm^{-1} and typical bands for C–F stretching around 1100 – 1000 cm^{-1} could also be clearly distinguished in the spectrum. In addition, the NMR spectra (Figure 1b) also confirmed the chemical structure of the synthesized monomer.

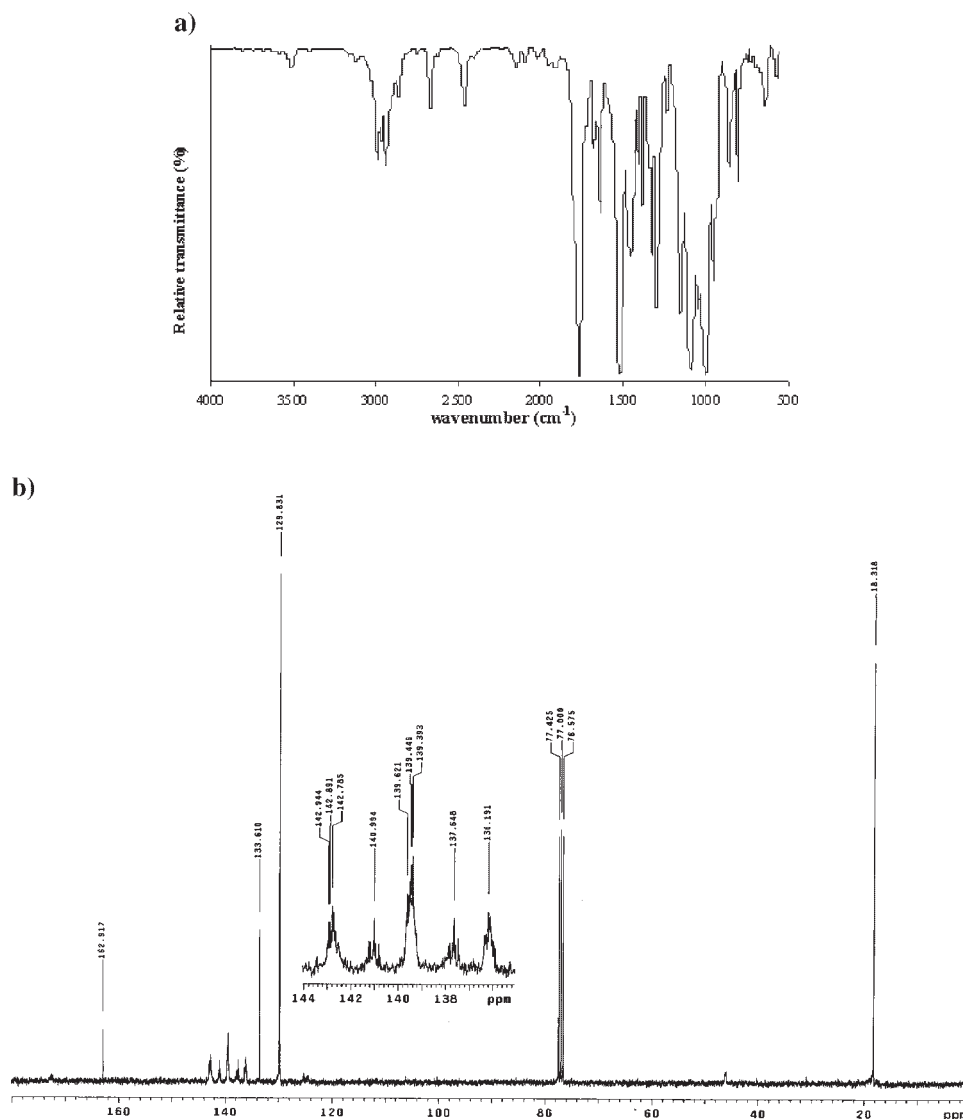


Figure 1. (a) IR and (b) ¹³C NMR spectrum of the synthesized PFM.

The proton spectrum showed a decoupled signal at $\delta = 2.03$ corresponding to the methyl group of the acrylated part of PFM and two signals at $\delta = 5.9$ and 6.4 from protons in the *cis* and *trans* position within this group. The carbon spectrum was set in accordance with those reported in the literature^[25] with a signal at 162.910 ppm that reflected formation of the ester bond between the acrylate and the phenol, a signal at ca. 139.6 ppm showing the C–F bond in the ring, two signals at 133.626 and 129.786 ppm that illustrate the presence of the double bond, and a signal at 18.272 ppm that reflects the presence of the CH₃ group.

Plasma Polymerization and Characterization of the Films

The deposition rate for bare PFM was 25 nm · h⁻¹. The deposition rate was 16 nm · h⁻¹ for the octadiene/PFM (6:1) copolymer and 8 nm · h⁻¹ for the divinyl ether/PFM (3:1)

copolymer, suggesting the different reactivity of the products used.

XPS widescan spectra of the PFM (10 W, duty cycle of 1/215 Hz, 2 h deposition) revealed the presence of oxygen, carbon, fluorine, and nitrogen. The C1s core level signals were fitted on various oxygen-containing functionalities and on fluorine-containing functionalities. Nitrogen functionalities were not considered, since Cl and N were found only in limited amount at the surface coating. Spectra were corrected for sample charging, setting the hydrocarbon signal to 285.0 eV, and the position of the others centroids accordingly adjusted. The XPS survey spectrum of the PFM polymer showed retention of fluorinated functionalities, but in levels below the theoretical ones, probably caused by fragmentation of the monomer during the polymerization. The XPS C1s spectrum of the polymer PFM showed the expected carbon environments such as C–R, COOR (R = C or H), and C–F, each exhibiting a characteristic chemical

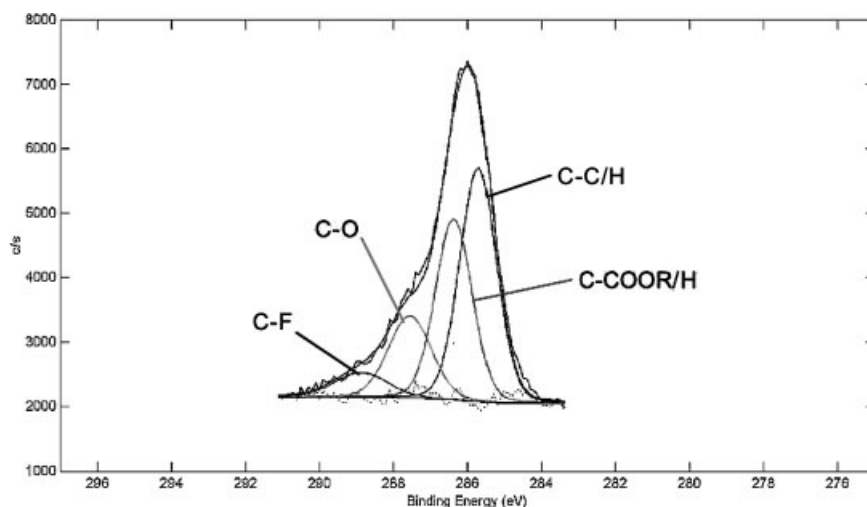


Figure 2. C1s core line fit for the PFM film, **A** C–C/H, **B**, C–COOH/R, **C** C–CO, **D** C–F.

shift (Figure 2). The spectrum was fit with four signals corresponding to hydrocarbon (C–C/H, A in the figure) set at 285.0 eV (see above), an α -ester carbon (C–COOH/R, B in the figure) at 285.6 eV, an ether carbon at 286.8 eV (C in the figure) and fluorinated carbon (C–F, D in the figure) at 288.2 eV. The existence of other oxygen-containing functionalities in addition to carboxylates constituted a further indication of monomer-oxygen reaction and fragmentation of the monomer in the plasma giving rise to the formation of side products during polymerization. A reaction between the film and the water desorbed from the walls of the plasma vessel (during polymerization), and atmospheric oxygen and water (after polymerization) might also have contributed.^[26,28]

XPS spectra of the PFM copolymerization with the two cross-linking copolymers (Table 1) showed the expected carbon environments, such as C–R, COOR and C–F, each exhibiting their characteristic chemical shift.

Carbon environments of the form C–R, C–O, C=O and COOR (R = C or H) and C–F were present in the XPS spectrum obtained from the polymer of PFM and 1,7-octadiene copolymerization, each exhibiting a characteristic chemical shift.^[28] This spectrum was fit with signals corresponding to hydrocarbon (C–C/H) set at 285 eV, an α -ester carbon (C–COOH/R) at 285.6 eV, an alcohol or ether carbon (C–O) at 286.7 eV, carbonyl (C=O) at 287.9 eV and carboxyl/carboxylate COOH/R at 289.2 eV. As expected, the amount of carbon was slightly higher than in the case of bare PFM (see Table 1), due to the presence of the two co-

monomers that are formed mainly by a hydrocarbon structure. Again in all cases, the existence of oxygen-containing functionalities in addition to carboxylates constituted a further indication of monomer-oxygen reaction and fragmentation of the monomer in the plasma, giving rise to the formation of side products during polymerization. A reaction between the film and the water desorbed from the walls of the plasma vessel (during polymerization), and atmospheric oxygen and water (after polymerization) have contributed to the composition of the coating.^[26,28] In the same way, the film obtained by copolymerization of PFM and divinyl ether showed a higher amount of oxygen (32.5% vs. 30.9% for bare PFM, see Table 1), attributed to the ether functionality in the molecule. Even in the case of the octadiene/PFM copolymer, the amount of oxygen compared to the bare PFM (33.1% vs. 30.9% for bare PFM, see Table 1) was higher. These results suggest that the use of the cross-linkers, octadiene and divinyl ether, promotes a higher oxygen incorporation into the film. The high loss of F amount in the divinyl ether/PFM polymer indicates the possibility of different polymerization mechanisms compared with the other polymerizations.

AFM Imaging

AFM analysis was performed for all samples. The AFM image of octadiene/PFM copolymer (Figure 3) showed flat, homogeneous surfaces, pinhole free with an average roughness of less than 0.5 nm (R_q) and a maximum

Table 1. Elemental analysis by XPS of the plasma polymerized monomers.

Monomer	%C	%O	%F	%N	C/F theoretical	C/F experimental
PFM	55.8	30.9	12.5	0.8	2.0	4.4
PFM/octadiene (1:6)	60.9	33.1	4.3	1.7	11.6	14.2
PFM/divinyl ether (1:3)	60.7	32.5	0.6	6.0	6.8	101.0

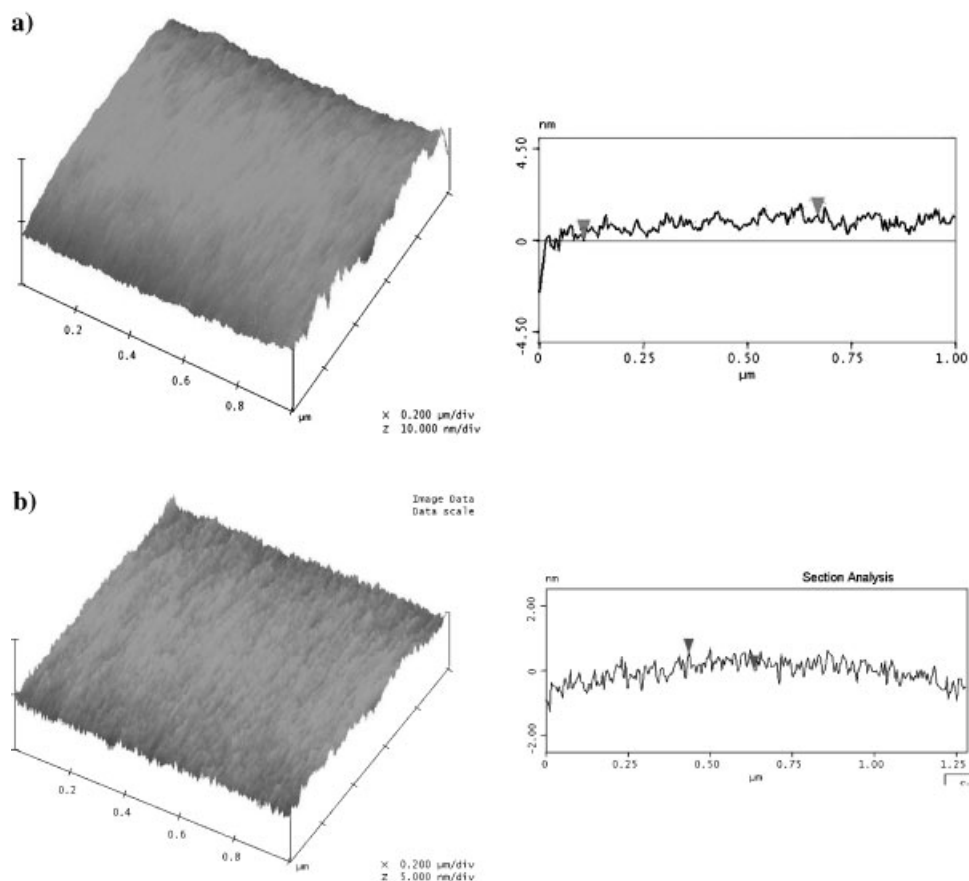


Figure 3. (a) AFM image and roughness profilometry corresponding to the octadiene/PFM copolymer film with a crosslinker/PFM ratio of 6:1, obtained under pulse plasma at 10 W, 15 Hz and a deposition time of 2 h, (b) AFM image and roughness profilometry corresponding to the divinyl ether/PFM copolymer film with a crosslinker/PFM ratio of 4:1, obtained under pulse plasma at 10 W, 15 Hz and a deposition time of 2 h.

roughness of 4 nm (R_{\max}). This low roughness is likely from a combination of two factors: pulsed plasma conditions, through which it is possible to control the ratio between nucleation and growth of the film with a slow deposition rate, and the geometry of the reactor. In fact, our research group has already reported this level of roughness in thiophene and pyrrole plasma polymerized films using conditions similar to those described in this paper.^[29]

Biotin and Streptavidin Linkage

A control sample (non-treated silicon wafer, Figure 4a) and PFM/octadiene-activated surfaces (Figure 4b) were exposed to a solution of fluorescein-conjugated streptavidin for microscopic fluorescence detection. Tween 20, a blocking agent composed of polyoxyethylene sorbitan monostearate that preferentially adsorbs to the background, was used to wash non-covalently bound biotin and to avoid non-specific adsorption of streptavidin.^[26] Compared to control sample (non-polymerized, Figure 4a), PFM/octadiene-activated surfaces effectively bound biotin, allowing self-assembly of

streptavidin, as shown by the fluorescence detection. This procedure establishes the basis for further attachment of other biotinylated biomolecules that enable control of cell attachment and proliferation.

Conclusion

PFM has been synthesized following a simple approach based on a general derivatization method for acrylates with a yield of 85%. The derivatized methacrylate was successfully used as monomer for plasma polymerization alone and copolymerized with different cross-linkers to improve the solvent resistance of the resulting deposited thin films. XPS analysis confirmed that labile ester groups remained on the functionalized surfaces. Such active esters enabled a fast reaction with amino-PEG biotin and subsequent self-assembly of streptavidin, a well-established platform for further immobilization of biomolecules that promote substrate-cell interaction.

Merging the versatility of plasma polymerization processes, via simple monomers and easy reaction conditions,

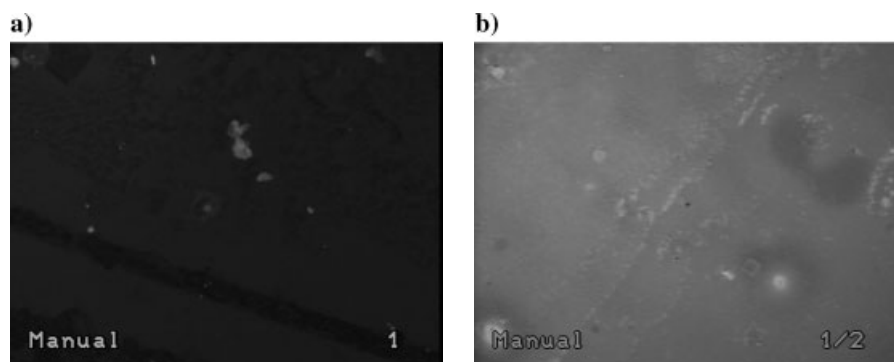


Figure 4. Fluorescence micrographs of a silicon wafer (a) and a silicon wafer after plasma polymerization of octadiene/PFM (ratio 6:1, obtained under pulse plasma at 10 W, 15 Hz, deposition time of 2 h) (b), both submitted to the treatment with biotin and fluorescein-conjugated streptavidin. Magnification is 50 \times .

with biological platforms that enable guidance of cell adhesion brings us closer to the ultimate goal of controlling cell function through structured surfaces for their application in tissue engineering.

Acknowledgements: E. E. and M. B. thank the *National Institutes of Health* (grants HL60407 and HL49039). E. G. thanks *Institut Químico de Sarria* for a Ph. D. fellowship.

- [1] A. Quintana, E. Raczka, L. Piehler, I. Lee, A. Myc, I. Majoros, A. K. Patri, T. Thomas, J. Mule, J. R. Baker, Jr., *Pharm. Res.* **2002**, *19*, 1310.
- [2] S. S. Mark, N. Sandhyarani, C. Zhu, C. Campagnolo, C. A. Batt, *Langmuir* **2004**, *20*, 6808.
- [3] R. Langer, D. A. Tirrell, *Nature* **2004**, *428*, 487.
- [4] S. F. Long, S. Clarke, M. C. Davies, A. L. Lewis, G. W. Hanlon, A. W. Lloyd, *Biomaterials* **2003**, *24*, 4115.
- [5] S. I. Jeong, S. H. Kim, Y. H. Kim, Y. Jung, J. H. Kwon, B. S. Kim, Y. M. Lee, *J. Biomater. Sci., Polym. Ed.* **2004**, *15*, 645.
- [6] H. H. Xu, D. T. Smith, C. G. Simon, *Biomaterials* **2004**, *25*, 4615.
- [7] T. Kokubo, H. M. Kim, M. Kawashita, *Biomaterials* **2003**, *24*, 2161.
- [8] Y. Liu, K. de Groot, E. B. Hunziker, *Ann. Biomed. Eng.* **2004**, *32*, 398.
- [9] J. Tessmar, A. Mikos, A. Gopferich, *Biomaterials* **2003**, *24*, 4475.
- [10] F. E. Black, M. Hartshorne, M. C. Davies, C. J. Roberts, S. J. B. Tendler, P. M. Williams, K. M. Shakesheff, S. M. Cannizzaro, I. Kim, R. Langer, *Langmuir* **1999**, *15*, 3157.
- [11] J. D. Whittle, N. A. Bullett, R. D. Short, C. W. Ian Douglas, A. P. Hollander, J. Davies, *J. Mater. Chem.* **2002**, *12*, 2726.
- [12] P. K. Chu, J. Y. Chen, L. P. Wang, N. Huang, *Mater. Sci. Eng., R: Reports* **2002**, *R36*, 143.
- [13] U. Vohrer, D. Hegemann, C. Oehr, *Anal. Bioanal. Chem.* **2003**, *375*, 929.
- [14] H. Schettlers, *Biomol. Eng.* **1999**, *16*, 73.
- [15] A. Scorilas, A. Bjartell, H. Lilja, C. Moller, E. P. Diamandis, *Clin. Chem.* **2000**, *46*, 1450.
- [16] Q. P. Qin, T. Lövgren, K. Pettersson, *Anal. Chem.* **2001**, *73*, 1521.
- [17] D. S. Wilbur, P. M. Pathare, D. K. Hamlin, P. S. Stayton, R. To, L. A. Klumb, K. R. Buhler, R. L. Vessella, *Biomol. Eng.* **1999**, *16*, 113.
- [18] K. Ohno, B. Levin, D. Meruelo, *Biochem. Mol. Med.* **1996**, *58*, 227.
- [19] Z. Yang, A. M. Belu, A. Liebmann-Vinson, H. Sugg, A. Chilkoti, *Langmuir* **2000**, *16*, 7482.
- [20] G. W. Cline, S. B. Hanna, *J. Am. Chem. Soc.* **1987**, *109*, 3087.
- [21] G. W. Anderson, J. E. Zimmermann, F. M. Callahan, *J. Am. Chem. Soc.* **1964**, *86*, 1839.
- [22] P. Ferruti, A. Betteli, A. Feré, *Polymer* **1972**, *13*, 462.
- [23] M. C. Tanzi, L. Rusconi, C. Barozzi, P. Ferruti, L. Angiolini, M. Nocentini, V. Barone, R. Barbucci, *Polymer* **1984**, *25*, 863.
- [24] J. Lahann, M. Balcells, T. Rodon, J. Lee, I. S. Choi, K. F. Jensen, R. Langer, *Langmuir* **2002**, *18*, 3632.
- [25] J. C. Blazejewski, J. W. Hofstra, C. Lequesne, C. Wakselman, U. E. Wiersum, *J. Fluorine Chem.* **1998**, *91*, 175.
- [26] J. Hyun, Y. Zhu, A. Liebmann-Vinson, T. P. Beebe, Jr., A. Chilkoti, *Langmuir* **2001**, *17*, 6358.
- [27] T. Lahtinen, E. Weselim, K. Rissanen, *New J. Chem.* **2001**, *25*, 905.
- [28] S. Fraser, R. D. Short, D. Barton, J. W. Bradley, *J. Phys. Chem., B* **2002**, *106*, 5596.
- [29] L. Martin, J. Esteve, S. Borros, *Thin Solid Films* **2004**, *74–80*, 451.