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Parylene C Surface Functionalization and Patterning with pH-Responsive Microgels

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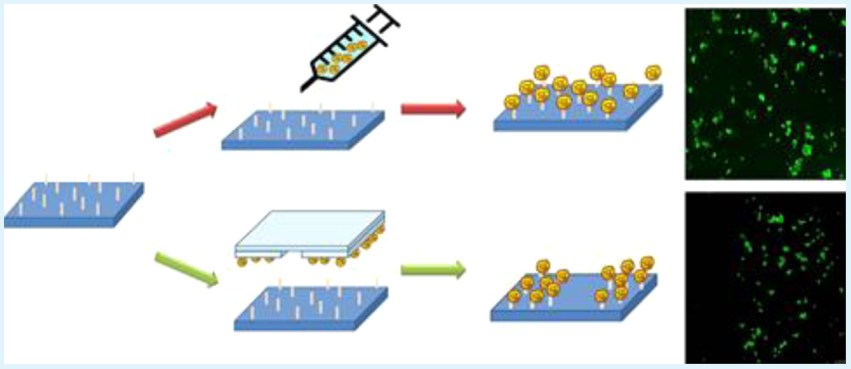
Supporting Information



ABSTRACT: Parylene C is a polymer well-known for its inertness and chemical resistance, thus ideal for covering and sealing 3D substrates and structures by conformal coating. In the present study, the Parylene C surface is modified by functionalization with pH-responsive poly(methacrylic acid) microgels either over the whole surface, or in a pattern through a poly(dimethylsiloxane) stamp. The surface functionalization consists of two phases: first, an oxygen plasma treatment is used to make the surface superhydrophilic, inducing the formation of polar functional groups and surface topography modifications; then, the plasma-treated samples are functionalized by drop casting a solution of pH-responsive microgels, or in a pattern via microcontact printing of the same solution. While both techniques, namely, drop casting and microcontact printing, are easy to use, fast, and cheap, the microcontact printing was found to provide a more homogeneous functionalization and to be applicable to any shape of substrate. The functionalization effectiveness was tested by the repeated uptake and release of a fluorescent labeled monoclonal CD4 antibody at different pH values, thus suggesting a new sensing approach.

KEYWORDS: *Parylene C, poly(methacrylic acid) microgels, pH-responsive, oxygen plasma, surface functionalization, patterning*

INTRODUCTION

Parylene is the general name of a family of polymers first discovered by Szwarc in 1947, as a product of the pyrolysis of *para*-xylylene.[1] Parylene C (pC), characterized by a chlorine atom substituted on the aromatic ring, combines chemical resistance and inertness with dielectric insulating properties, low permeability to corrosive gases and moisture, and other useful properties in many fields. In addition, its conformal deposition via Room Temperature—Chemical Vapor Deposition (RT-CVD)[2] produces a transparent, flexible, and chemically inert layer, available at room temperature and suitable as a coating for three-dimensional (3D) structures in a wide range of applications, such as chemical sensors,[3,4] microelectrodes,[5] and antiadhesion layers in microfluidics.[6] More recently, pC conformal coating has been used as an encapsulation method for smart implantable microelectromechanical systems (MEMS) devices,[7] and for controlling the mechanical and sensory properties of artificial hair cells in mechanical and flow sensors.[2,8] Moreover, thanks to its stability to most chemical solvents and biological fluids, pC has emerged as potential

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material for biomedical applications, such as medical endoscopes and tools or prostheses.[9] Furthermore, flexible, textile and stretchable electronics represent emerging research areas for Parylene use.[10]

Parylene inertness is an obstacle for all the applications that require chemical functionalization or interaction with cells or biomolecules.[11−13] So far, many attempts have been made in order to modify the pC surface properties (i.e., surface chemistry or wettability properties);[14−17] however, only a few methods, physical or chemical, have succeeded. Among them, the Friedel−Crafts acylation, although able to create carbonyl groups on the pC surface further exploitable to induce other functionalities,[18,19] exhibits some limitations such as time-consuming procedures and the use of hazardous chemicals. Other strategies consist of surface roughening,[20] ultraviolet (UV) light irradiation[21] and plasma or electron beam bombardment.[22] Plasma surface treatment has been attracting a lot of interest as it allows fast processing. In fact, the exposure of the surface to ionized gases can give rise to various phenomena: etching, implantation, activation, passivation, cross-linking of surface molecules and functionalization.[23−29] It causes a physical and/or chemical modification of the surface thus inducing modifications in the wettability properties. Such modifications may be used to enable further functionalization steps on pC surface.

In the present study, after a preliminary plasma treatment, a pC functionalization with micrometer-sized poly(methacrylic acid) (PMAA) hydrogels (microgels) is achieved in order to obtain a smart pC surface, suitable for diagnostic, monitoring, therapeutic and sensor applications. The functionalization of the pC surface with pH-responsive microgels merges the desirable properties of the former (transparency, flexibility, resistance and conformal deposition) with the responsiveness of the latter, thus allowing for the prospective fabrication of biosensors for liquid environments (body fluids, environmental fluids, foods).

PMAA microgels are hydrogel networks exhibiting the unique property of pH responsiveness, as they undergo to conformational and volume variation as the pH turns from acidic to basic and vice versa. In this way, microgels can be controlled to encapsulate and subsequently release macromolecules, diagnostic agents and even bioactive molecules.[30,31] Furthermore, their microscale dimensions are compatible with miniaturized sensors and lab-on-chip devices, and provide large surface to volume ratio as well as faster time response.[32,33] Specifically, two methods for functionalizing the highly inert pC surface with pH-responsive microgels are introduced and compared: the first is functionalization by drop casting a solution of microgels onto the pC samples, while the second entails microcontact printing (μCP)[34−36] to induce patterns of microgels. Both methods include a preliminary oxygen plasma treatment in order to switch the pC wettability character from hydrophobic to superhydrophilic upon creation of hydrophilic groups on the surface.[15,16,22] The two methods are compared by confocal microscope analysis and by testing the capability of PMAA microgels to uptake/release a fluorescent labeled antibody after their covalent immobilization on pC surface.

Both approaches are promising tools for the realization of smart pC surfaces that can find application in biosensing and diagnostics. Due to the enormous changes in the properties of hydrogels,[37] they have been successfully employed in many chemical sensor systems.[38] In particular, devices based on stimuli-responsive hydrogels, able to swell and deswell in response to a change in their surroundings, i.e., solvent composition, temperature, and pH, can show a high sensitivity and selectivity. To the best of our knowledge, it is the first time that the pC surface has been functionalized with pH-sensitive PMAA. Microgels volume dependence from pH is a promising feature for application in pC-coated MEMS. A remarkable example thereof is constituted by miniaturized mechanical transducers in a liquid environment, like microcantilevers and bending plate sensors,[39] where the resistant and insulating coating provided by pC is necessary.

EXPERIMENTAL SECTION

Materials. Silicon wafers type (100), 525 μm thick, used as substrates for the subsequent treatments, were purchased by Okmetic.

Granular Parylene C poly(*para*-xylylene) was supplied in form of powder dimers by Specialty Coating Systems.

PMAA microgels were synthesized according to the procedure reported in literature and exhibit a size in the range of 3−6 μm.[32] The description of PMAA microgel synthesis is reported in Supporting Information section.

Poly(dimethylsiloxane) (PDMS) (Sylgard 184 Silicone Elastomer), employed for the replicas of the silicon master for μCP experiments, was purchased from Dow Corning Corporation and supplied in two compounds: a prepolymer and a cross-linker.

The sample washing was carried out in purified water through a Milli-Q Plus system.

The FITC-anti CD4 monoclonal antibodies were supplied by Sigma-Aldrich and employed to test the preserved functionality of the PMAA microgels.

Parylene C Deposition Process. Parylene C, a soft polymer material, belonging to the family of the poly(*para*-xylylene) polymers, is characterized by the substitution of one hydrogen atom of the aromatic monomers with a chlorine one. The pC film is deposited onto silicon samples of 1 cm^2 by means of a RT-CVD conformal coating (Specialty Coating Systems, PDS 2010 Labcoater system model).

The deposition process starts with the powdered dimers, which vaporize at a temperature in the range of 100−150 °C and a pressure of 1 Torr and are reduced in monomers. Subsequently, at a temperature of 650−700 °C and at a pressure of 0.5 Torr, the gaseous monomers pyrolize and polymerize. A further, simultaneous decrease in temperature (20−25 °C) and pressure (0.1 Torr) in the deposition chamber makes the deposition of the polymer film possible. This process leads to a conformal coating on any substrates type and morphology. To deposit a 1 μm thick layer of pC, an amount of about 1 g of dimer powder is required.

Plasma Oxygen Surface Treatment. The oxygen plasma surface treatment was carried out in a two-parallel-plate plasma system (March Instruments Corporation, PX250). The reactor has a volume of about 0.016 m^3, the plate sides are 17 and 20 cm, and their distance is around 5 cm. Several power values (25, 50, 75, 100 W at a frequency of 13.56 MHz) were tested for different time intervals (1, 2, 5, 10 min), with a constant pressure of 300 mTorr.

PMAA Microgel Deposition on Oxygen Plasma Treated Parylene C. The PMAA microgel deposition is performed immediately after the oxygen plasma surface treatment, following two alternative procedures: deposition by solution drop casting and microcontact printing (μCP) from PDMS stamps on selected areas where the stamp touches the pC surface.

The deposition via drop casting is achieved by dropping a solution of PMAA microgels (6.2 mg/mL) 2-(*N*-morpholino) ethanesulfonic acid buffer (referred to as MES buffer) (1 mM) onto the oxygen plasma treated pC samples, completely covering them, with few drops of 45 μL each one. The solution is left to dry out overnight at room temperature, and the samples are then carefully washed with distilled water, dried under nitrogen stream and stored at 4 °C.

For microcontact printing of microgels, a mold silicon master (1.5 cm long, 700 μm wide and 25 μm high), prepared by defining a

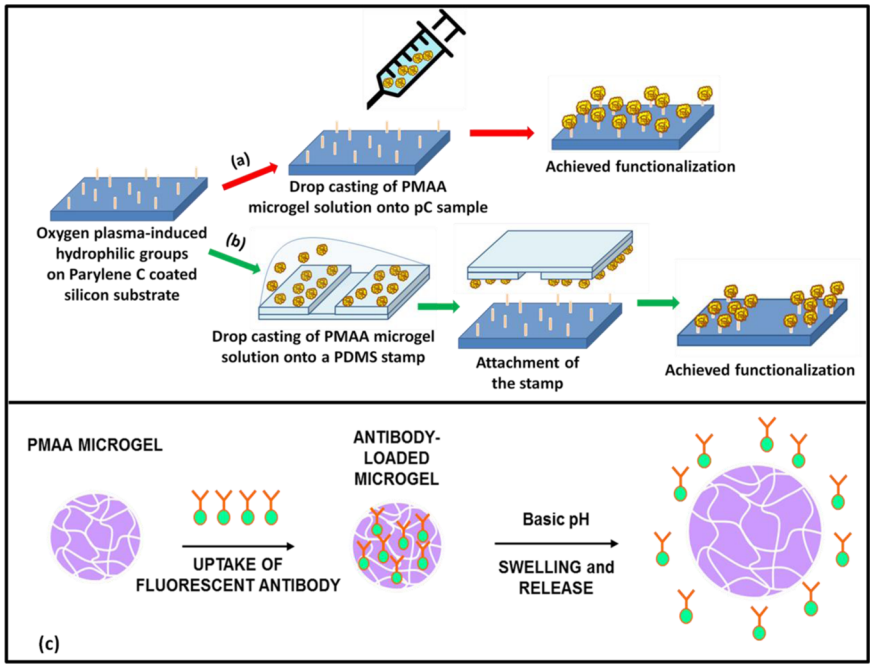

Figure 1. Schematic representation of the two different methods for the surface functionalization with PMAA microgels: (a) drop casting treatment of the surface; (b) microcontact printing of a pattern on the surface. (c) A schematic representation of a microgel, labeled molecules, and uptake/release processes at different pH values is shown.

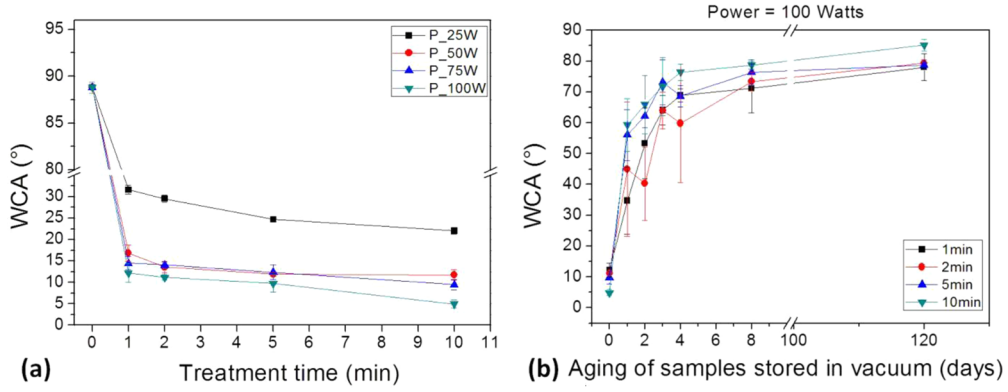

Figure 2. (a) The four curves in the graph show the drastic reduction of the measured WCAs as a function of the plasma oxygen treatment time, for different values of applied plasma power. (b) Aging (hydrophobic recovery) effect of plasma treated pC surfaces. All the measurements refer to samples treated with 100 W power for different process times.

negative relief of the microchannel pattern via SU-8 photolithography on silicon wafer, is used to fabricate PDMS stamps (10:1 (w:w) ratio of prepolymer and cross-linker). After a curing step of 5 min at 110 °C, the PDMS stamps are hydrophilized with an oxygen plasma treatment[40] (100 W, 300 mTorr) for 10 min, to better accommodate the same as before hydrophilic solution of PMAA microgels (6.2 mg/mL) in MES buffer (1 mM) drop-casted on them. After casting the PMAA microgel solution on the PDMS stamps, the latter are applied onto the pC sample surfaces, previously treated with plasma, and left adhering overnight before detachment. The samples are abundantly washed with deionized water and stored at 4 °C.

A schematic representation of the two strategies of functionalization is available in Figure 1a,b.

Uptake and Release Experiments. After the functionalization processes, the pH-responsiveness of PMAA microgels has been tested using a labeled biomolecule. PMAA hydrogel is a carbon-based network bearing on the surface carboxylic groups that dissociate according to their acid equilibrium. Indeed, at higher pH values, the Coulombic repulsions between the deprotonated carboxylic groups lead to a considerable swelling of the microgels, whereas under acidic conditions, the carboxylic groups are not ionized and the microparticles are in a collapsed state. The uptake of fluorescein isothyocianate (FITC)-labeled monoclonal CD4 antibody into the PMAA microgels, immobilized on chemically modified pC surfaces, is carried out by incubating the microgel-functionalized surface on a rotary mixer for 4 h at room temperature (RT). Soon after, the sample loaded with the labeled antibody is rinsed thoroughly with distilled water to remove the free labeled antibodies, and finally dried under nitrogen flow.

In detail, the antibody solution is first diluted with (MES buffer solution) (1 mM) to a final concentration of 5 μg/mL, and thereafter, the uptake experiments were carried at pH 5.0−5.5 in order to exploit the ion-exchange mechanism.[32] The unloading experiment is carried out by immersing the loaded pC samples in solution of MES buffer at pH 8.5. The basic pH induces the microgel swelling after its deposition on Parylene surface[41] and therefore the release of the encapsulated material.

The pH value of the incubating solution is set by adding submicroliter amounts of 0.5 M NaOH and 0.5 M HCl.[42,43] A

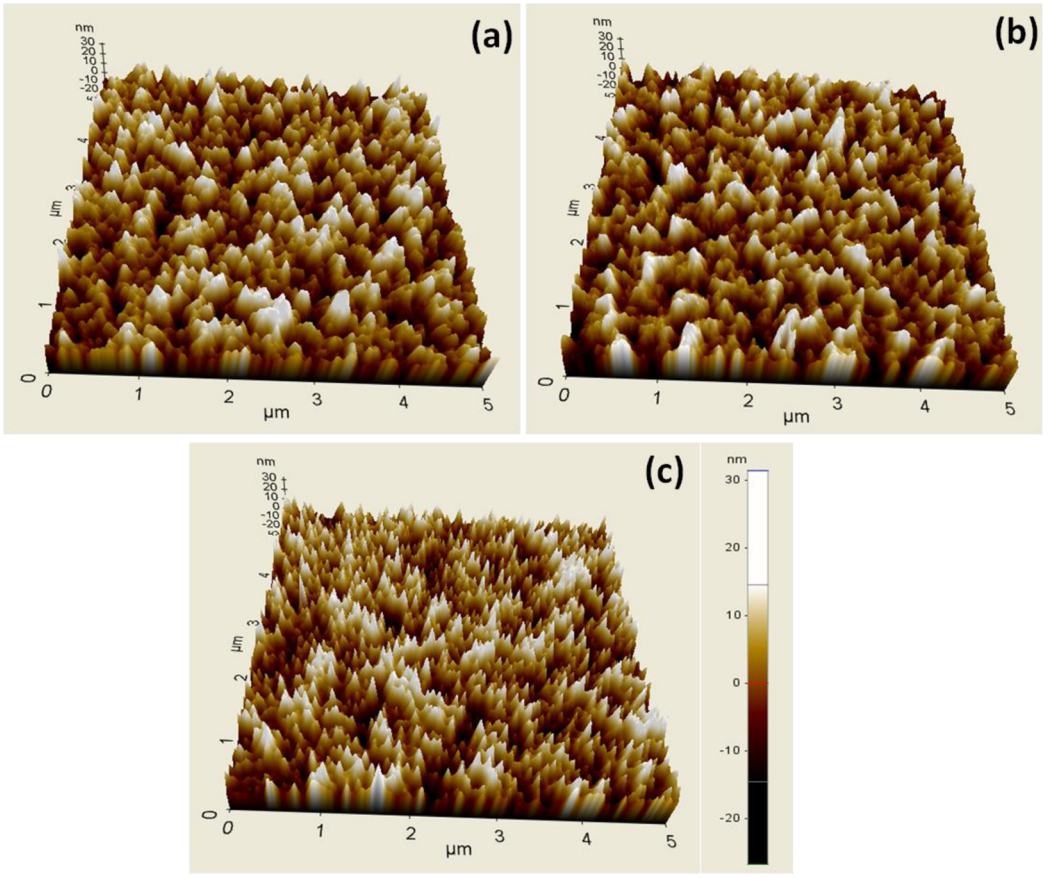

Figure 3. AFM images of (a) untreated pC sample, (b) 10 min-50 W treated sample, and (c) 10 min-100 W treated sample. Surface area to volume ratios for the three samples are 1.13 ± 0.01, 1.50 ± 0.01, and 1.89 ± 0.01, respectively. In (c) the surface area increases by 67% compared to (a) and by 26% compared to (b).

schematic representation of the uptake/release processes of labeled biomolecules at different pH values is available in Figure1c.

Sample Characterization. The thickness of the deposited pC on the surface of the silicon substrates is measured by a Bruker Dektak Xt profilometer.

Immediately after oxygen plasma treatment, the water contact angle (WCA) of the pC coated surfaces is measured with a KSVCAM200, Kruss, Germany, contact angle goniometer, using distilled water droplets of 2 μL. The average contact angle values with corresponding standard deviations are reported for each sample based on 5 measurements.

Non-Contact mode Atomic Force Microscope (NC-AFM, XE-100 PSIA) analysis is conducted to monitor topography modifications induced onto pC surfaces by oxygen plasma treatment. The Root Mean Square Roughness and the surface area to volume ratio of AFM images are calculated on the basis of three independent measurements.

To evaluate surface morphology features, a grain/pore analysis of AFM images is performed by SPIP software (The Scanning Probe Image Processor, by Image Metrology). The analysis provides the segmentation by means of a threshold method that detects peaks and valleys in the image and, for each peak, geometrical parameters such as perimeter, area and volume (Table S1, Supporting Information).

The uptake and release processes of labeled biomolecules are analyzed by laser scanning confocal microscopy. Confocal microscopy measurements were performed at room temperature by using a confocal microscope (FV-1000 Olympus) in epilayer configuration.

RESULTS AND DISCUSSION

Parylene C exhibits a WCA of 88.8 ± 0.7°, resulting largely hydrophobic.[44] Its wettability properties can be modified by means of appropriate treatments in both the directions, in order to induce a well-defined hydrophobic or hydrophilic character.

To make it hydrophilic, 1 cm^2 silicon substrates coated with 1 μm of pC were processed with oxygen plasma, at varying pressures and treatment times. WCA analysis was performed to characterize the best combination of plasma parameters for rendering Parylene superhydrophilic. Figure 2a shows the obtained results, revealing that, at constant treatment time, increasing the plasma oxygen power from 25 to 100 W, leads to a considerable enhancement of the surface hydrophilicity; in addition, increasing the treatment time makes the pC surface hydrophilic, as WCAs are much smaller than 60°. Finally, at the maximum power value (100 W), the two longest treatments (5 and 10 min) induce superhydrophilicity, with WCAs equal to 9.8 ± 2.1° and 4.9 ± 1.0°, respectively. This is also true for the samples treated for 10 min with 75 W power and for 10 min with 50 W, showing very low WCAs, respectively 9.5 ± 1.2° and 11.7 ± 1.2°, typical of a superhydrophilic behavior. To study the aging of the samples made hydrophilic, pC samples treated with maximum power and for different process duration were stored in vacuum and analyzed. WCA was monitored for a period of 4 months. The graph in Figure 2b shows a recovery of the samples, as WCA values saturate toward the initial almost hydrophobic behavior.

Plasma induced hydrophilicity is generally due to the formation of chemical groups containing oxygen, such as carbonyl, aldehyde carboxyl groups, or hydroxyl groups, which determine the increase of the surface energy.[15,16,22] XPS

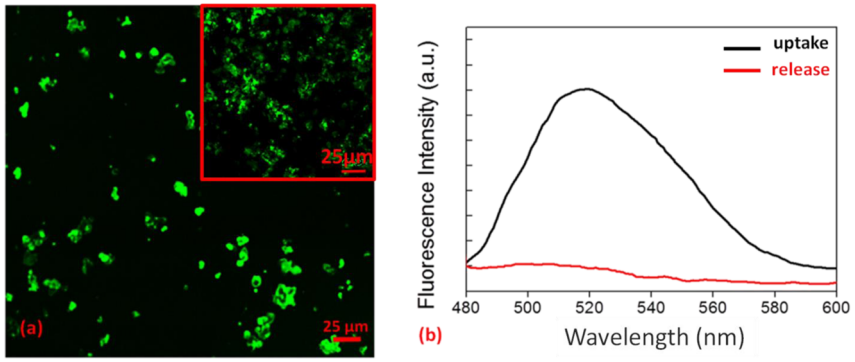

Figure 4. (a) Confocal microscopy image of pC sample functionalized with PMAA microgels via drop casting, in which the sample exhibits the FITC-labeled antibody successfully loaded at pH 5.0−5.5. In the inset, a portion of the same sample is shown where aggregates of microgels are visible. (b) Fluorescence spectra of the immobilized microgels after the uptake (black curve) and the release (red curve) of the FITC-labeled antibodies.

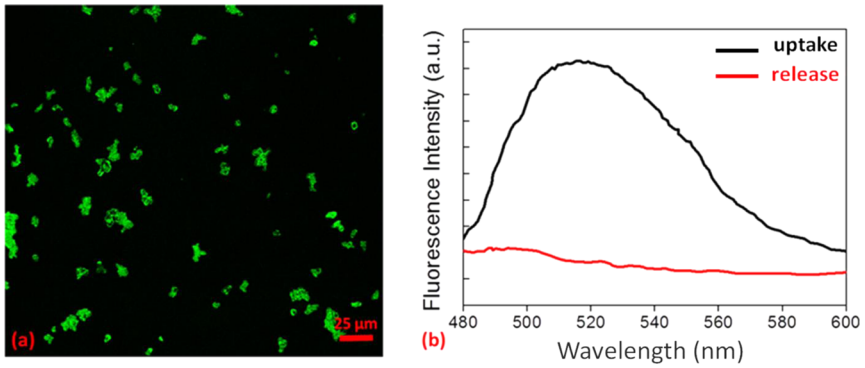

Figure 5. (a) Confocal image of pC sample functionalized with PMAA microgels via μCP treatment showing the uptake process of FITC-labeled antibody. (b) Fluorescence signals of FITC-labeled antibodies corresponding to the uptake (dark spectra) and the release (red curve) experiments.

analysis reported in Hoshino et al. and Seong et al.[13,45] on plasma oxygen treated hydrophilic pC samples reveals a larger content in oxygen atoms and a reduction in carbon ones. Hydrophilicity is enhanced by plasma induced roughening (often referred to as nanotexturing) as suggested by Tsougeni et al.,[28] leading to superhydrophilicity as predicted by the Wenzel equation. However, hydrophilicity is quickly lost due to hydrophobic recovery (aging effect). The aging effect (Figure 2b) suggests, as already observed in a previous work,[15] that reactions between free hydrophilic groups and air molecules/contaminants reduce the surface energy to values of WCA very close to the initial one. The larger standard deviation in WCA measured during aging (Figure 2b) indicates that the hydrophobic recovery is not homogeneous over the whole sample surfaces, thus suggesting complex dynamics acting on the polymer surface.[46,47] In the case of the most hydrophilic sample (100 W, 10 min) the hydrophobic recovery is studied also in water medium (Supporting Information Figure S1).

A morphological analysis performed by AFM, is carried out in order to verify the topographical effects caused by oxygen treatments. Moreover, this characterization allows to understand whether the superhydrophilicity effect is not only due to the chemical effects (oxygen-based chemical groups) but also to plasma-induced topographical effects (enhanced roughness).[27−29] Specifically, the two superhydrophilic samples processed for 10 min, respectively, at 50 and 100 W, are investigated and compared to a control sample (untreated pC). The results, shown in Figure 3, reveal that the samples treated for 10 min at 50 W and the control sample have a similar surface topography, while the one treated for 10 min at 100 W shows sharper and smaller surface features leading to an increased surface area. Nevertheless, the parameters that quantify the surface roughness, such as the Root Mean Square Roughness (R_{rms}), are similar for all the three samples. R_{rms} is indeed equal to 5.7 ± 0.3, 5.3 ± 0.2, and 5.4 ± 0.3 nm, respectively, while the surface area to volume ratio is 1.13 ± 0.01, 1.50 ± 0.01, and 1.89 ± 0.01, showing a significant increase in the exposed surface area for the plasma treated samples. The most heavily treated sample (100 W, 10 min) exhibits the most featured surface and the smallest WCA, due to an increased exposed surface and consequently an higher density of plasma-induced hydrophilic groups thereon.[15,16,22,28,44−49]

Detailed analysis of the surface using SPIP image analysis software is shown in Supporting Information Table S1.

The functionalization of the pC surface with pH-responsive PMAA microgels was carried out on the most hydrophilic sample (100 W, 10 min), because of the high density of the just mentioned hydrophilic groups.

As previously stated, two different methods are tested to attach microgels to the pC surface: coating by drop casting, and the second one by μCP, by using a PDMS stamp soaked with the microparticle solution.

To check whether the different functionalization routes succeeded, all the treated samples were immersed in a solution of FITC-anti CD4 antibody. The evidence that the PMAA microgels are successfully deposited on the pC surface and that their typical pH-responsive behavior is preserved was provided

by the pH-triggered uptake and release of the FITC-labeled antibody. Both processes were monitored by LSCM.

We found that the drop casting treatment permits an efficient functionalization of the pC surface, as shown in Figure 4a, where it is possible to see microgels marked by the fluorophore. The size of the fluorescent spots is of a few micrometers, except for some aggregates visible on the surface, comparable to that of the synthesized microgels. The PMAA microgel density is $3.3 \times 10^{-4} \pm 2.9 \times 10^{-4}$ particles/μm^2. The distribution of microgels onto the surface is not always homogeneous, as some aggregates can be formed, mainly related to the evaporation of the solvent (inset in Figure 4a). At the same time, Figure 4b shows the fluorescence spectra of immobilized microgels after the uptake process (black curve) and the release (red curve), which demonstrate that the functionality of the PMAA microgels is preserved.

Microcontact printing is also promising for the surface modification of pC; indeed, the obtained results (Figure 5) demonstrate a high efficiency of functionalization. In this case, the used stamp applies an external mechanical force facilitating the PMAA microgels to be mechanically transferred onto the pC surface through the contact between the two different surfaces.[50,51] The PMAA microgel density is $6.9 \times 10^{-4} \pm 2.6 \times 10^{-4}$ particles/μm^2, and in particular, microgels are more homogeneously distributed onto the polymer substrate and only few and small aggregates are visible. The percentage of particles transferred from the PDMS stamp to the parylene surface was calculated, resulting to be approximately 90%.

There are some different but synergic effects underlying the proposed functionalization methods, which lead to microgels adsorption onto the pC surface. They mainly consist in the creation of weak, noncovalent bonds (i.e., van-der-Walls interactions between carbon atoms of both pC substrate and PMAA network and hydrogen bonds) along with electrostatic affinity between the plasma-induced hydrophilic groups on the pC surface and the hydrogels.[52,53] Despite the intrinsic weakness of the individual binding mechanisms, microgels are firmly anchored to the surface also after the hydrophobic recovery of pC, and they preserve their pH-dependent functionality in loading−unloading FITC-labeled antibodies for at least 3 uptake−release cycles performed over a total time interval of 2 months. Moreover, after each process of uptake or release, microgels resisted to the washing under a deionized water flow of 0.8 mL/s and to the subsequent drying with nitrogen flow (0.5 L/s), before being stored at 4 °C.

It is worth remarking that the simple alternative to the aforementioned methods, the overnight immersion of the plasma treated pC into a vessel containing microgel solution, resulted ineffective in attaching PMAA microparticles to the surface, even with 10× and 100× higher concentrations.

The microcontact printing functionalization technique permits the surface patterning according to the shape of the PDMS stamp, with a sporadic formation of small aggregates, as visible in Figure 6. In the figure, it is possible to unambiguously conclude that the functionalization takes place only on the printed side of the sample. μCP permits to pattern planar and bent substrates with a fine geometrical control because the elastomeric stamps can adapt to virtually any kind of surface; moreover PDMS stamps are generally highly inert, durable, reusable and can be exploited for a big variety of target molecules. The patterning resolution is in this case limited by microgel dimensions; at the best, it is potentially reaching a minimum of few micrometers.

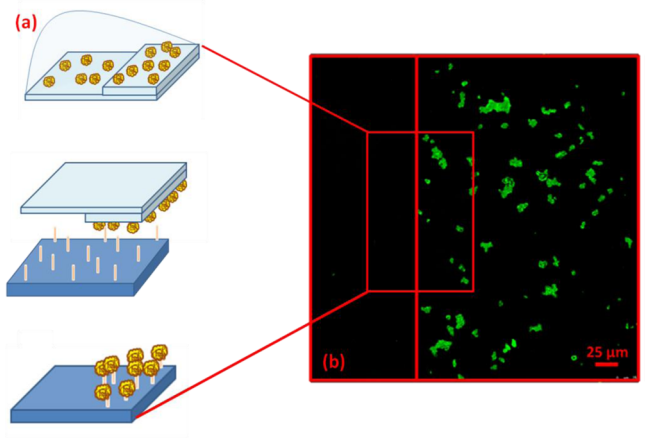

Figure 6. (a) Schematic representation of the μCP technique steps to obtain a substrate functionalized only on one side. (b) Fluorescent confocal images of a pC coated silicon sample functionalized with microgels by means of the μCP technique. It is evident how the functionalization happens only on the right side of the PDMS stamp.

CONCLUSIONS

In the present study, Parylene C (pC), known to be a highly inert material, has been successfully functionalized with micrometric pH-responsive poly(methacrylic acid) (PMAA) hydrogel particles. A preliminary plasma oxygen treatment has been optimized to make pC superhydrophilic and thus susceptible to further chemical modifications. Two different strategies for the functionalization have been identified and compared: the first one consisting in drop casting a solution of microgels onto the pC sample surface, and the second one consisting in microcontact printing the same solution. They are both cheap, easy to handle, highly reproducible, and neither is time nor reagent consuming. Microcontact printing provides a more homogeneous functionalization of the sample surface, characterized by less and smaller aggregates, and it is applicable in a desired pattern to a substrate independently from its surface morphology. The pH-responsive hydrogel functionalization, leading to load and release of a big variety of solutes upon varying the pH values of the surrounding solution, is promising as a responsive platform for many applications involving pC coated devices, such as mechanical biosensor and Lab-on-chip or biomedical devices. PMAA hydrogels functionalization on Parylene sealed and water-proofed MEMS opens a new range of applications where pH sensing can be coupled to piezoresistivity and piezoelectricity-based strain sensing. This combination allows the realization of microarrays biosensors with a compact nonoptical detection, individually addressable by functionalization through single drop-casting or microcontact printing.

ASSOCIATED CONTENT

Supporting Information

Synthesis of PMAA microgels, analysis performed on AFM images by means of SPIP software, aging effect (hydrophobic recovery) of oxygen plasma treated parylene C samples stored in pure water and in vacuum. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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