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ARTICLE *in* LANGMUIR · APRIL 2001

Impact Factor: 4.46 · DOI: 10.1021/la0016930

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Microfluidic Networks Made of Poly(dimethylsiloxane), Si, and Au Coated with Polyethylene Glycol for Patterning Proteins onto Surfaces

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Received December 4, 2000. In Final Form: April 17, 2001

Microfluidic networks (μFNs) are passive (self-filling) devices incorporating microchannels for guiding minute volumes of fluids over surfaces. μFNs can be employed to localize the deposition of proteins from aqueous solutions onto substrates, for example. The walls of the channels must be hydrophilic for this purpose and should ideally resist the adsorption of proteins. We made μFNs using poly(dimethylsiloxane) (PDMS), Si/SiO_2, and Au-covered Si and derivatized them with poly(ethylene glycol)s (PEGs) to fulfill both of these requirements. The grafting of the PEG molecules is optimized for either type of μFN: the networks from PDMS and silicon are derivatized using PEG-silanes and the Au-coated networks are derivatized with a thiolated PEG. Additionally, the zones of the Au-covered Si μFNs separating the channels are selectively covered with a hydrophobic thiol using microcontact printing. X-ray photoelectron spectroscopy and contact angle measurements indicate that all grafted layers have the expected chemical composition and are thin, homogeneous, and hydrophilic where desired. Finally, using fluorescently labeled antibodies we show that these μFNs are more effective for patterning, with high positional accuracy and edge resolution on PDMS substrates, than conventional O_2-plasma-treated μFNs made from PDMS. Overall, our approach should help in making and using μFNs made from different materials but having similar surface properties.

Introduction

Patterned deposition of biomolecules onto surfaces is a prerequisite for the development of array-based biosensing devices.[1,2] Microfluidic networks (μFNs) represent a compelling approach for the patterning of biological molecules,[3,4] because these devices can guide solutions of proteins conveniently over regions of a substrate to localize the adsorption of proteins.[5−10] There are several requirements concerning substrates and μFNs for a successful patterning of proteins:[4,6] (i) μFNs must be sufficiently hydrophilic to promote filling of the microchannels by capillary action, (ii) the contact between the substrate and μFN should be good enough to seal the channels, (iii) promoting the flow of a large volume of solution inside the microchannels can be necessary to guarantee a sufficient supply of proteins, and (iv) the μFN should have protein-repellent surfaces to prevent unproductive loss of proteins to the walls of the microchannels. The first two requirements are mandatory, whereas the latter two are desirable.

In this article, we present a method for tailoring the surface properties of several types of μFN to fulfill the above-mentioned requirements. This extends the possible scope of applications of μFNs and makes them more convenient to use. Several combinations of materials for the substrate and for the μFN are possible, to ensure conformal contact between them and thus effective sealing of the channels. Either the μFN or the substrate can be an elastomer, for example. Figure 1 illustrates three of these possibilities, which we have investigated in detail and discuss here. μFNs made from poly(dimethylsiloxane) (PDMS) are soft and can be used on many substrates, regardless of their mechanical properties. Conversely, μFNs made in “hard” Si and Au can be used to pattern proteins onto PDMS substrates.[9] Harder μFNs are advantageous because they are mechanically more stable than μFNs in PDMS[11] and hence offer greater design possibilities. None of these three materials has ideal chemical surface properties to be readily used as μFNs, however. PDMS is a hydrophobic material, and therefore a μFN in PDMS must be hydrophilized before its channels can be filled by capillary action.[4] A brief oxygen-plasma treatment can be sufficient for this purpose. But in this case, the μFN must be used immediately after the plasma treatment or kept under water; the hydrophilized surface is not stable in ambient but reconstructs or contaminates toward a hydrophobic surface.[12,13] Si, with its native oxide, and Au have polar, wettable surfaces when they are clean,

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10.1021/la0016930 CCC: $20.00
Published on Web 06/02/2001

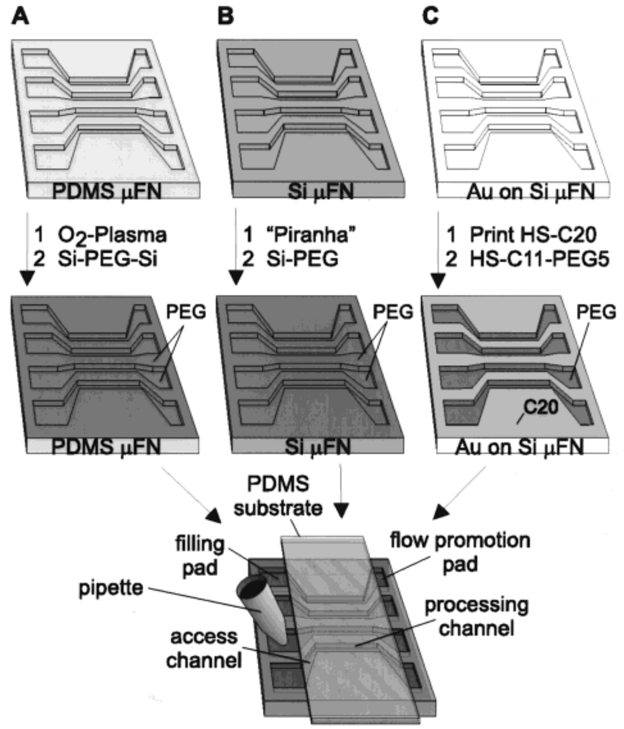

Figure 1. Strategies for coating μFNs made from different materials with poly(ethylene glycol)s. (A) The surface of μFNs in PDMS is oxidized in a O_2-based plasma and grafted with a PEG−disilane. (B) μFNs in silicon are cleaned with a strong oxidizing solution and silanized with PEGs. (C) HS−C20 is microcontact-printed on the raised parts of Au-coated μFNs, and the channels are derivatized with PEGs to control selectively the protein repellency and wetting characteristics of this type of μFN. All μFNs have the same design and are used in a similar way by placing a PDMS substrate across the channels.

but in practice these materials tend to contaminate under ambient conditions to become hydrophobic and chemically less defined. The possible loss of proteins because of their adsorption onto the walls of the μFNs during the patterning step is a second concern. Ideally, proteins should be spent only to derivatize the substrate, especially because, typically, a limited amount of proteins is displaced through the microchannel.

We choose to derivatize either type of μFN with functionalized poly(ethylene glycol) (PEG) polymers to yield μFNs with wettable and protein-repellent surfaces.[13−16] Derivatizing PDMS and Si/SiO_2 was done in an analogous manner. μFNs in PDMS were treated first with an O_2 plasma before grafting a poly(ethylene glycol) di(triethoxy)silane (Si−PEG−Si) layer to it (Figure 1A), and μFNs in Si/SiO_2 were cleaned with a strong oxidizing solution and grafted with 2-[methoxy-(polyethyleneoxy)$_{6-9}$-propyl]-trimethoxysilane (Si−PEG)[17] (Figure 1B). In the case of Au-coated Si μFNs, we combined self-assembly and microcontact printing of thiols:[1,18] Eicosanethiol (HS−C20) was printed[9,19] onto the raised structures (gaps separating the channels) of the μFNs, which come into contact with the substrate during patterning.[19] Subsequent immersion of the printed μFNs in a solution of 11-penta(ethyleneoxy)undecanethiol (HS−C11−PEG5) localized the self-assembly of this hydrophilic, protein-repellent thiol[20,21] to the inner walls of the microchannels (Figure 1C).

Experimental Section

2.1. Proteins and Chemicals. 2-[Methoxy-(polyethyleneoxy)$_{6-9}$-propyl]-trimethoxysilane (Si−PEG, MW of 460−590, purity > 90%) from Gelest (Tullytown, PA), poly(ethylene glycol) di(triethoxy)silane (Si−PEG−Si, MW ~ 3400) from Shearwater Polymers (Huntsville, AL), and rabbit anti-guinea-pig IgG-TRITC from Sigma Chemie (Buchs, Switzerland) were used as supplied. Deionized water (R > 18.2 MΩ cm^{-1}) produced with a MilliQ purification unit (Millipore, Boston, MA) was used. All other chemicals were products of Aldrich (Milwaukee, WI).

2.2. Fabrication of μFNs and Substrates. PDMS μFNs resulted from curing PDMS (Sylgard 184, Dow Corning, Midland, MI) at 60 °C for at least 24 h against a fluorinated Si master. The fabrication of Si μFNs is described in detail elsewhere.[22] PDMS μFNs were oxidized in an oxygen plasma ($P(O_2) \sim 0.36$ mbar, 140 W coil power, Technics Plasma 100-E, Florence, KY) for 10 s. The networks were immediately immersed in the Si−PEG−Si solution (1 mM Si−PEG−Si in water containing 0.8 mL concentrated HCl per liter) for 2 h at room temperature (RT). Afterward, the PDMS μFNs were washed twice in water and sonicated in water for 2 min. Si μFNs were sonicated in ethanol/water (1:1) for 5 min, dried, and then cleaned and oxidized with "piranha" solution (30% H_2O_2 and concentrated H_2SO_4, 1:4 vol; caution: strong oxidizing agent) for 10 min. The networks were washed three times with water, sonicated in water for 10 min, blown dry and immediately immersed into the silane solution. The grafting was performed in a 3 mM solution of the Si−PEG in toluene containing 0.8 mL concentrated HCl per liter for 18 h at RT. The networks were washed in toluene (1×), ethanol (2×), and water (2×) and sonicated in water for 2 min to remove nongrafted material. Au-coated μFNs were fabricated by evaporating 1 nm of Ti and 100 nm of Au onto Si μFNs using an e-beam evaporator (Edwards FL 400). A hydrophobic monolayer was self-assembled on top of the raised structures of the Au-coated μFNs by printing for 10 s HS−C20 (0.5 mM solution in ethanol for the ink) using a (flat) PDMS stamp. These μFNs were immersed subsequently into a solution of HS−C11−PEG5 (5 mM in ethanol) for 10 s to form PEG monolayers exclusively inside the channels and pads. All μFNs were used with flat PDMS substrates. The PDMS substrates were cleaned with sonication in ethanol/water (1:1) for 3 min and then rinsed in water and dried under a stream of N_2 prior to their patterning.

2.3. X-ray Photoelectron Spectroscopy (XPS). XPS spectra were acquired on a Sigma Probe VG Scientific spectrophotometer operating at a base pressure of $<10^{-9}$ mbar and equipped with a monochromatized Al Kα source (E =1486.6 eV). The X-ray spot was focused down to 300 μm. The analyzer had an angle of 45° to the sample, and samples were mounted on a multisample holder stage for examination under the same conditions. Spectra are referenced to the O 1s peak at 532 eV or to the Au 4f$_{5/2}$ peak at 84 eV, alternatively. For all samples, survey spectra were acquired first with a pass energy of 80 eV (0.2 eV steps for 40 ms), and two high-resolution spectra for N 1s taken with a pass energy of 40 eV were averaged (0.05 eV steps for 100 ms). The electron beam used to generate the X-rays had in all cases an intensity of 6.0 mA, which remained stable within <5% during the experiments. The intensity of the peaks from the substrates did not vary noticeably during the experiments, which indicated no particular damages on the surface of the grafted samples during the measurement. XPS on PDMS was done using a flood gun (~0.6 μA emission current) at a partial pressure of Ar of

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$\sim 5 \times 10^{-8}$ mbar for charge compensation of these insulating samples. Charge compensation for measuring on the Si substrate was not necessary because the oxide of this substrate was relatively thin (2.0 nm as measured with ellipsometry). The sensitivity of the XPS is independent of the type of substrate when charge compensation is effective and when the experimental conditions are conserved between samples. All PDMS samples were cut to a size of <40 mm^2 to minimize deterioration of the ultrahigh vacuum environment. The X-ray and flood guns were switched on, to warm them, before introducing the samples in the analysis chamber, and the X-ray gun was carefully degassed before acquiring the spectra to prevent contamination by the PDMS samples.

2.4. Contact Angle Measurements. Wettability of the modified surfaces by water was determined using a Krüss contact angle goniometer (Hamburg, Germany) equipped with a motorized pipet (Matrix Technology, Nashua, NH). Advancing and receding contact angles were measured at several spots on each sample and the respective values were averaged.

2.5. Fluorescence Microscopy. Fluorescence images of the tagged antibodies present on the surface were acquired with a fluorescence microscope (Nikon Labophot-2) equipped with a charge-coupled device camera (ST-8, SBIG, Santa Barbara, CA) cooled to −5 °C. The fluorescence images were up to 765 × 510 pixels (16 bits dynamic range) and were acquired with 4 s integration time and a magnification of ∼10, using software designed for this camera (e.g., SkyPro, Software Bisque, Golden, CO).

2.6. Protein Adsorption Assay. PDMS substrates were placed onto the channels of the μFN. A 1 μL drop of protein solution was pipetted onto the filling pad and transported through the channel by capillary action. After a 10 min incubation time, the substrate was removed under buffer (phosphate buffered saline, PBS) and rinsed with PBS and water.

Results and Discussion

Our strategy to prepare wettable and protein-repellent μFNs is to graft a layer of PEGs onto the surface of PDMS, Si, and Au μFNs. This strategy is practical because PEGs are relatively chemically inert and thus compatible with a large variety of anchoring groups. Grafting PEG chains onto PDMS is the only case in this strategy requiring a pretreatment of the μFN surface with an O_2-based plasma. This plasma treatment oxidizes the surface of PDMS and produces a thin, glassy silicate layer on the PDMS, which renders it suitable for the grafting of silane-derivatized PEGs. In the case of PDMS, the grafting of a PEG−disilane molecule prevented the strong loss of hydrophilicity of the O_2-plasma-treated μFNs, by highly mobile silicon rubber chains.[13] This loss of hydrophilicity, termed "hydrophobicity recovery", has been studied in detail.[23,24] The possible causes for the hydrophobicity recovery of oxidized PDMS are mainly (i) the migration of low molecular weight siloxane chains and monomers from the bulk to the surface, (ii) the reorganization of the interface to expose less hydrophilic species at the PDMS−air interface in order to lower surface free energy, and (iii) accumulation of airborne hydrophobic contaminants. The possibility of having cross-linking between the silane anchoring groups did not seem to disturb the attachment of the PEG layer onto the surface, at least not in the concentration and under the conditions of grafting that we used. Furthermore, the relatively large molecular weight (3400 g mol^{-1}) of the PEG molecule grafted onto μFNs made of PDMS may help to stabilize the grafted surface against reconstruction toward a more hydrophobic surface. We studied the hydrophobicity recovery of PDMS grafted with a series of PEGs with molecular weights ranging from 500 to 5000 g mol^{-1} and having one or two trialkoxysilanes[25] and found that Si−PEG−Si was probably the best type of PEG to graft to PDMS; this PEG is commercially available and preserves the hydrophilicity of the oxidized PDMS surface for over 3 weeks.[13]

The XPS survey spectrum in Figure 2 corresponding to this grafting reaction is largely consistent with previous work[16] and with the expected chemical composition of the PEG−PDMS surface. This spectrum and those obtained for the other types of μFNs were obtained by focusing the X-ray beam in the middle region of the filling pads and also between the two filling pads of the C20-coated part of the Au μFN; the C 1s peak reveals the presence of oxidized carbon atoms at 286.5 eV and represents ∼30% of the C 1s signal found in Au−PEG. Such oxidized carbon species are typically absent from PDMS surfaces treated with plasma conditions similar to ours.[13,24,26] The O 1s peak does not provide a major indication on the composition of the grafted layer because the oxidized PDMS substrate should contain oxygen atoms in a variety of chemical environments, which overlap with the oxygen signals from the PEG layer. As for oxygen, the Si peaks are dominated by the signals from the substrate. Importantly, the C 1s signal attributed to PEG is relatively small compared to that of PEG on Si/SiO_2 or Au, which suggests that the PEG layer on PDMS is thin or corresponds to an incomplete monolayer. This might result from a lower density of silanol anchoring groups on oxidized PDMS than on SiO_2 or from the condensation of silanols after the plasma treatment.[23,26] The recovery of hydrophobicity, starting after the plasma treatment, may also account for hiding some silanols from the anchoring groups of PEGs. The delay between plasma oxidation of PDMS and grafting with PEGs was kept to a minimum for this reason.

The oxide of a μFN made in Si reacts similarly with silanes as O_2-plasma-treated PDMS, but the excellent homogeneity and chemical stability of this interface facilitates grafting silanes. For this reason, we used a relatively short monosilane having 6−9 PEG units in the case of Si/SiO_2, for which the resulting grafted layer had the expected chemical composition, as shown in Figure 2. Two species for Si are visible, which correspond to Si from the bulk of the wafer and, at a slightly higher binding energy, to Si from the thin native oxide. These signals mask the signal associated to the Si anchoring group, which is otherwise attenuated by the presence of the monolayer. The O 1s peak from the PEG is similarly overwhelmed by the oxygen from the substrate. The C 1s signal at 287 eV corresponds to carbon from the PEG layer.[21] The intensity of this C 1s peak suggests the presence of a thicker PEG layer on Si than on Au. This is consistent with the work by Papra et al.,[17] in which the same compound was grafted onto Si/SiO_2 wafers under equivalent conditions and in which the PEG had a measured thickness of ∼1.6 nm, whereas the type of PEG that we grafted to Au is composed of an alkyl chain ∼1.2 nm long and has a PEG moiety of ∼1.3 nm.[21]

Au-coated μFNs offer an advantage over μFNs in PDMS or Si/SiO_2: Au can be selectively microcontact printed with one type of alkanethiol, leaving unprinted regions of the μFN available for the chemisorption from solution of a second type of alkanethiol. HS−C20 was selected because it is simple to microcontact print onto Au and it forms a highly hydrophobic surface thereupon. We note that the

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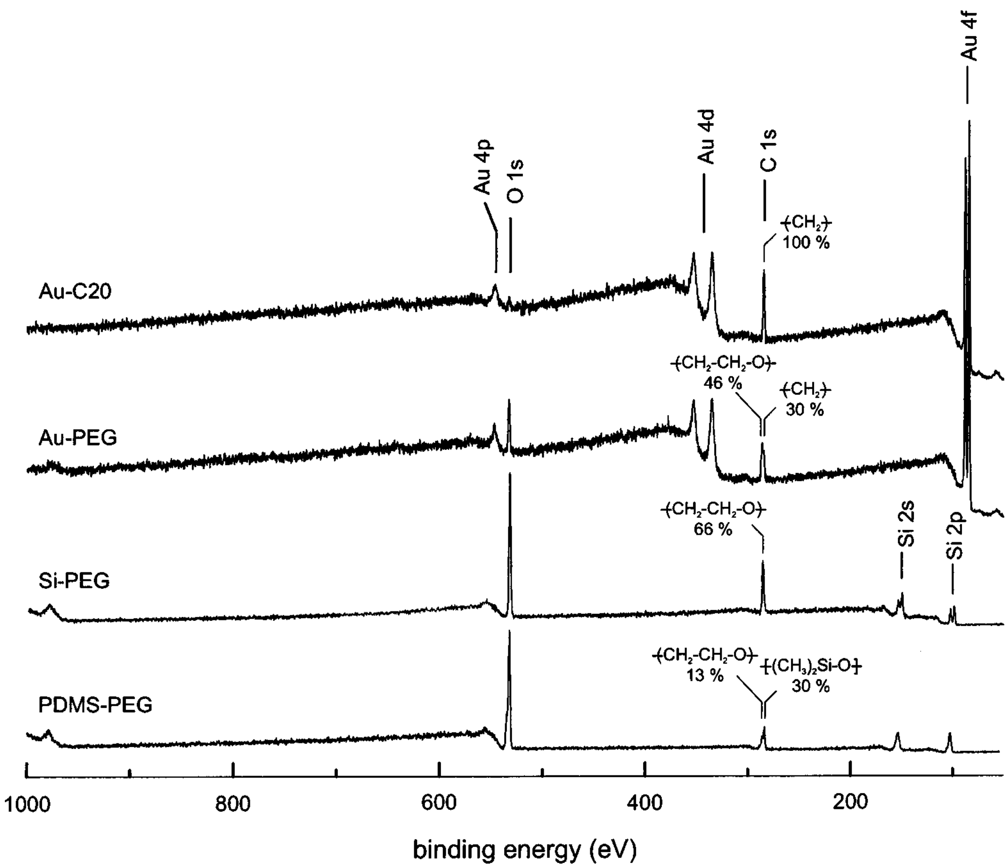

Figure 2. XPS overview spectra measured on the surface of μFNs derivatized with specific films. The XPS spectra on the Au μFNs were obtained inside the filling pad (Au−PEG) and between two filling pads (Au−C20). Information on the C 1s peaks obtained from high-resolution spectra are reported on the survey and compared to the C 1s signal measured on Au−C20, which can serve as a reference.

small O 1s peak in the Au−C20 spectrum (Figure 2) was the only unexpected element detected in the XPS spectra. This oxygen might correspond to a small quantity of HS−C11−PEG5 molecules that were possibly exchanged with some of the printed HS−C20 molecules during the self-assembly from the solution of the PEG-derivatized monolayer. We used a relatively high concentration of HS−C11−PEG5 solution and a short reaction time when chemisorbing those PEG molecules, to minimize this exchange of thiols.[27] The C 1s and O 1s peaks are both associated with the grafted PEG layer. The C 1s peak has one component at 284.6 eV, corresponding to carbons from the alkyl chains, and a second component at 286.8 eV, corresponding to the carbon atoms linked to the oxygen forming the PEG units.[27]

Contact angle measurements with water as the probe liquid were used to measure the hydrophilicity of the μFNs and are reported in Figure 3. The advancing contact angles of water reflect the susceptibility of either type of μFN for filling, whereas the hysteresis between advancing and receding contact angles gives an indication of the chemical and/or topological heterogeneity of the μFN surface and therefore indirectly reveals the quality of the coating.[28,29] All contact angles were measured on zones of the μFNs that were planar and large enough to avoid contact

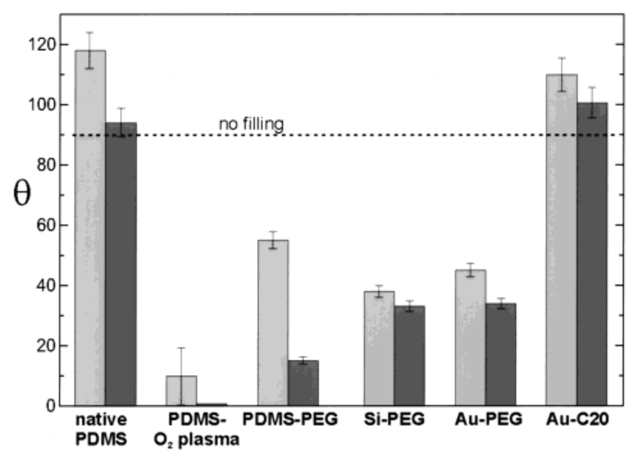

Figure 3. Advancing (bright columns) and receding (dark columns) contact angles of water (captive drop method) on surface-treated μFNs. All contact angles were stable in time, except those of PDMS treated only with an O_2 plasma, and measured 5 min after the plasma treatment. Advancing contact angles must be lower than 90° to permit filling of the microchannels with solutions of proteins, owing to capillary pressure. Capillary pressure increases with diminishing contact angles.

between water droplets and microstructures, which would have influenced the wetting behavior of the probe liquid. Native PDMS is evidently a hydrophobic material; thus, untreated PDMS microchannels cannot be filled by aqueous protein solutions. In contrast, PDMS, Si, and Au μFNs each with grafted PEG have a comparable hydrophilicity. The advancing contact angles of ~40−50° observed for these surfaces indicate that it will be easy

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to fill these μFNs with solutions of proteins. The larger hysteresis between the advancing and the receding contact angle observed for the PDMS−PEG surface reveals that this μFN has a less chemically homogeneous and/or morphologically smooth surface than μFNs in Si or Au; PDMS is the product of a chemical reaction involving prepolymers of various chain lengths and degree of functionality, filler materials, catalysts, and modulators, and the plasma treatment creates microcracks on the surface of the oxidized polymer.[30] PDMS freshly treated with O_2 plasma only is more hydrophilic than PDMS grafted with PEG. We observed, however, that the microcracks produced by the plasma on the surface of the oxidized PDMS accelerate the hydrophobicity recovery when μFNs are stressed too much during handling.[30,31] Alternatively, the very low advancing contact angle with water of plasma-treated μFNs in PDMS might lead to the excursion of some sample solutions to adjacent channels during filling of the μFN pads.[22] HS−C20 self-assembled on Au has a similar hydrophobicity to that of PDMS and is very effective in preventing the excursion of liquid away from the fillable parts of the network.

The PEG-coated μFNs were used to pattern fluorescently tagged IgGs from solution onto PDMS substrates (Figure 4). The concentration of proteins ranged from 400 down to 2 μg mL^{-1}. This range of concentrations was selected to evaluate the practical limits for patterning proteins onto PDMS using these μFNs. The patterning parameters (duration of deposition, substrate, and rinsing steps, for example) and the geometry of the μFNs were kept the same during all experiments to facilitate comparing the performances of either type of μFN. We examined the fluorescence and the N 1s XPS signal associated to the proteins on the substrate as well as on the walls of the μFNs (unproductive deposition). No fluorescence signal could be recorded from the hard μFNs because of quenching (n.a. in Figure 4). As can be seen for all the μFNs, the amount of proteins deposited on the substrate is proportional to their concentration in solution, and for the detection conditions of the fluorescence used here, a significant amount of proteins is detected on the surface even for the 20 μg mL^{-1} solution. The contrast and resolution of the pattern are high in all cases but are probably the best for the Si and Au μFNs. The images obtained on the PDMS μFNs hydrophilized with an O_2 plasma and coated with PEG reveal a loss of IgGs in the microchannel which was filled with the most concentrated solution of proteins. When considering the fluorescence signal on the PDMS substrates, it becomes clear that these losses are only moderate and that these μFNs were overall efficient in repelling proteins from their walls. As a complement to the fluorescence experiments, we studied the loss of protein on all the μFNs by recording the regions corresponding to N 1s peaks using XPS (Figure 4). The PDMS μFN treated with an O_2 plasma and the PEG-coated PDMS μFN feature an important difference: the surface of the plasma-hydrophilized μFN is not stable[26] but recovers its original hydrophobicity of PDMS over time. This hydrophobicity recovery poses practical problems because it is easily accelerated by flexing and handling the μFN and because some time is needed to position the μFN over the substrate and to fill it. As a result, the μFN can quickly become less protein-repellent while it recovers its hydrophobicity. We estimate from the N 1s XPS signals that the amount of proteins deposited onto the μFN in PDMS, used 1 min after the plasma treatment, corresponds to ~9% of a full monolayer of IgGs. This amount increases up to ~16% and ~20% when the delay between the plasma treatment and use of the μFNs increases to 5 and 20 min, respectively. In contrast, we found that the PEG-treated μFNs remained hydrophilic for more than 3 days,[25] while conserving a high degree of protein repellency. The amount of IgG lost to this type of μFN is between 6% and 8% of a full monolayer. The XPS N 1s regions for the Si and Au PEG-coated μFNs corroborate the indications given by Figures 2 and 3: grafting a PEG layer on these surfaces was not only simpler than grafting it on a PDMS elastomer, but it resulted in an even better protein-repellent layer. There is no trace of nitrogen found by XPS in the N 1s region of the PEG−Si μFN, and although there seems to be a trace of nitrogen in the N 1s signal for the Au−PEG type of μFN, this signal is too small to be quantified.

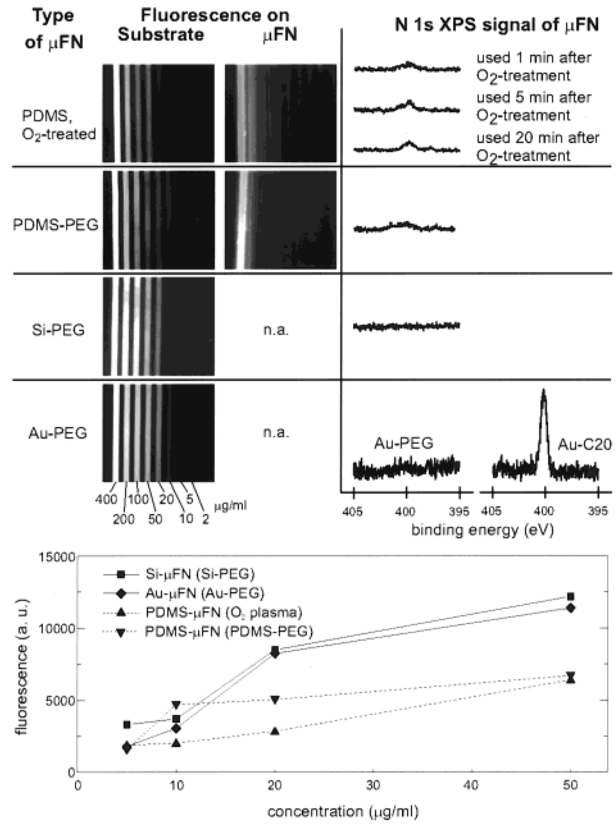

Figure 4. Fluorescence microscopy and XPS reveal the amount of TRITC-tagged antibodies deposited from solutions with decreasing concentrations on a PDMS substrate (images in left column) or lost on the walls of the μFNs (images in right column) used for this patterning. Fluorescence signals could not be measured on the Au and Si μFNs because of strong quenching of the fluorescence, but the N 1s XPS signals reveal the quantities of proteins lost on all types of μFNs. The XPS signals measured on the Au μFN are displayed enlarged compared to the other signals. The amount of protein lost on the O_2-plasma-treated μFN increases with the delay between the plasma treatment and use of the μFN. The lower graph quantifies the surface fluorescence signal associated to the deposited IgGs depending on their concentration in solution and the type of μFN used.

The overall amount of protein lost to the walls of the different types of μFN might not appear very critical, at least not for depositing proteins from concentrated solutions. This may not be the case when the solutions of proteins are diluted and flow in relatively long microchannels.[6] The graph in Figure 4 which shows the surface fluorescence associated with the immobilized IgGs, depending on their concentration in solution and the type

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of μFN used, corroborates the previous observations: for a concentration equal to or less than 50 μg mL^{-1}, the amount of protein deposited onto the substrate using μFNs in PDMS is already significantly lower than when Si−PEG and Au−PEG μFNs are used. Finally, all fluorescence values for even lower concentrations converge toward the background level for this surface fluorescence assay.

Conclusion

The networks of three types of μFNs including an elastomer (PDMS replica), an oxide (micromachined Si/SiO_2), and an oxide covered with a metal (Au) were made hydrophilic and protein-repellent by derivatizing their channels with PEG moieties. This expands the scope of applications of μFNs for the patterning of proteins onto substrates without requiring very specific surface treatments of the different types of μFNs. As PEG molecules are protein-repellent in general, we think that our grafting strategies can be successful for a large variety of proteins. Preventing or minimizing losses of proteins because of their deposition onto the channels of a μFN adds to the versatility of PEG-coated μFNs. Because it is possible to use an elastomer such as PDMS for the substrate to pattern the deposition of proteins, the preparation of "hard" μFNs in Si might prove particularly attractive. Indeed, the coating of such μFNs with an evaporated Au film seems ideal, because it opens the possibility to tailor independently the surface properties of the μFN inside the channels and in the sealing regions.

Acknowledgment. We thank H. Wolf, C. Donzel, I. Caelen, J. Ph. Renault, H. Schmid, F. Kamounah, and K. Schaumburg for their help and discussions, R. Widmer and U. Drechsler for their help in preparing the microfluidic networks, and P. F. Seidler for his continuous support. This work was supported in part by the Swiss Federal Office of Education and the BIOTECH European project BIOPATT (BIO4CT980536).

LA0016930