Tailormade Microfluidic Devices Through Photochemical Surface Modification

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A new pathway for the generation of polymer-based microfluidic devices with tailor-made surface chemistry is described. A simple photochemical process is used to covalently bind polymer molecules to the surfaces of microchannels fabricated by hot embossing. The substrates for the embossing process have the format of a compact disk (CD). CDs from polymethylmethacrylate and polyethylene-co-norbornene were chosen due to their good optical properties. Thin films of polymers containing photoactive benzophenone units were deposited onto the surface of the thus generated devices. These films were subsequently irradiated with UV light leading to the surface-attachment of ultrathin polymer networks. In contrast to their unmodified peers, the obtained, modi-fied microfluidic channels coated with hydrophilic, photoattached layers can be filled in a straightforward manner with water by capillary forces. Channels coated by thin films of poly(ethyloxazoline) show complete resistance to non-specific protein binding. Generation of hydrophobic patches inside the modified microfluidic channels using benzophenone-containing fluoropolymers allows the generation of passive micro-fluidic valves to direct fluid motion in these CD-based devices.

Introduction

Microfluidic devices are becoming increasingly popular and widely integrated into a broad spectrum of analytical applications ranging from chip-based capillary electro-phoresis and CD-based total bioanalytical systems to lab-on-chip devices.[1–8] Most of the microfluidic devices that were originally developed are based on glass,[9–13] and Si/SiO$_2$[12] as conventional photolithographic processes can be used to obtain the required microfluidic design in these materials. Additional advantages of these inorganic sub-strates are high electroosmotic flow owing to their inherent hydrophilicity and their surface charge, transparency to visible light (glass), and high thermal stability. Transpar-ency is for a variety of applications an important issue as it allows direct visual inspection and optical readout of the analytical process.

With these advantageous features, however, also certain drawbacks of both materials come along such as high fabrication cost, high sealing temperatures, high

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background fluorescence, and strong unspecific protein adsorption when in contact with biological fluids.[13] To avoid some of these problems, recently polymers have become more and more attractive candidates for materials used for microfluidic devices especially polydimethylsiloxane (PDMS), polymethylmethacrylate (PMMA), polystyrene (PS) polyethylenetetraphthalate glycol (PETG), polyvinylchloride (PVC), and polycarbonate (PC).[14–27] Advantages of using polymer substrates compared to their inorganic peers are their tunable optical, mechanical, and surface properties. The most attractive feature, however, is the ease of processing of such materials which allows a facile fabrication of complex structures at high speed and low cost.

A comprehensive review of the vast number of materials and systems is beyond the scope of this article and the reader is referred to the literature.[28]

As far as microfluidic devices with good optical properties are concerned, the choice of materials is rather restricted and essentially reduced to a relatively small pool of possible materials; the key polymers in that respect are PMMA and PMMA based copolymers,[20,22–27] polycarbonates of various composition[19,21] and, as a new class of polymers, polyethylene-norbonene copolymers (cyclic olefine copolymers, COCs),[4,5,29–32] which are obtained by metallocene polymerization.[33] With regard to the former two classes of materials the thermal stability of the devices is a concern for many applications. Polymers from the previously mentioned classes have only moderately high glass transition temperatures (T_g),[34] which are even lower in the presence of water. Upon exposure to water the polymers become slightly swollen and water acts as a plasticizer lowering considerably the glass transition temperature. Thus, if the polymers become exposed to aqueous (buffer) solutions as commonly used in biooriented applications, the systems are prone to mechanical deformations well below the glass transition temperature commonly found in reference tables. In our own experiments we observed that if a PMMA sample was heated in aqueous buffer solution close to the boiling point the sample deformed under its own weight and became significantly warped (data not shown).

The latter material mentioned above, the polyethylene copolymer, has a high T_g (around 140 °C depending on the composition and formulation of the polymer)[15] and is not plasticized by water. It has excellent optical properties, as it is an inherently amorphous material with very high transparency and low background fluorescence. The down side, however, for biooriented applications is that it is strongly hydrophobic. It is not easily wetted by water, which is generally not advantageous for microfluidic applications.

One way to overcome wetting problems is the chemical modification of the surfaces involved. As wetting is of critical importance for many (especially polyolefin) applications a large potpourri of techniques have been developed. Surface modification of polymers has been accomplished by the use of high corona discharge techniques,[35] flame or plasma-treatment,[36–38] or treatment with strong oxidizing or reducing agents.[39] In many cases the harsh treatment leads to strong degeneration of the polymeric substrates, rendering them unusable for delicate bioanalytical purposes.[40] On the other hand, in all cases, where the generation of thick, degraded layers on top of the material is avoided, the induced surface hydrophilicity is usually only short lived. This is especially well known for flame-, corona and plasma treated surfaces and is commonly named ''hydrophobic recovery.''[35] Hydrophobic recovery is a thermodynamically driven process, which is due to the fact, that the surface free energy of a material can be lowered considerably if non-polar groups are brought to the surface. As the surface reconstruction is caused by small-scale local movements of surface groups it is usually relatively fast and thus almost impossible to avoid. In many cases within minutes or hours a hydrophobicity almost similar to that before the surface treatment is reached, which severely limits the shelf life of devices treated this way.[41,42]

In the past, we have described a new process, which allows to photochemically attach polymers to surfaces.[43,44] During this process polymers containing photoactive benzophenone moieties are simultaneously crosslinked and attached to the substrate surface. In this work, we report on the use of polymers containing such photoactive groups along the chain to modify the surface of polymeric microfluidic devices. We describe the photo-induced attachment of hydrophilic as well as hydrophobic polymers to the polymer substrates with the aim to obtain microfluidic channels with tailor-made properties. Special focus is placed on the protein adsorption properties on the obtained surfaces as this will be a critical factor for introducing such microfluidic devices into bioanalytical applications.

Experimental Part

Materials and Methods

(1H,1H,2H,2H-Perfluorodecyl)acrylate (Aldrich, 97%) (PFA) was purified chromatographically over neutral aluminum oxide, distilled under vacuum from copper(I) chloride, and stored at (−30 °C). Poly(methylmethacrylate) ($\overline{M}_n = 100\,000$, PDI = 1.15) was obtained from PSS Polymer Standards Service. 1,1,2-trichlorotrifluoroethane (TCTFE, Fluka) and all other reagents were used as received. Benzophenone groups containing methacrylate (MA-N-BP) polymers shown in Figure 1 were synthesized from partially hydrolized (upto 12.3%) polyethyloxazoline according to previously published procedures.[45]
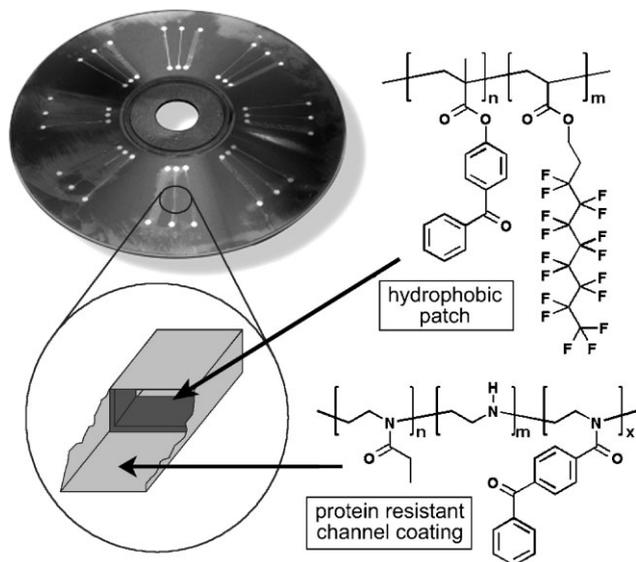
Figure 1. Schematic description of the surface modification of microfluidic channels.

Ellipsometric measurements were performed on a DRE–XO2 C ellipsometer operating with a 638.2 nm He/Ne laser at an incident angle of 70°. For data analysis it was assumed that the refractive indices of the polymer films are the same as those of the bulk material. Surface plasmon resonance (SPR) measurements were performed in an attenuated total reflection (ATR) configuration where s- or p-polarized laser light (He/Ne, 632.8 nm) is coupled into a 50–70 nm gold film deposited on the substrate slides using a glass prism (LaSFN9; $n = 1.842$), as schematically depicted in Figure 2. The reflected light intensity as a function of the angle of incidence was detected by a photodiode.

Synthesis of a Benzophenone Containing Fluoropolymer

3.27 g (6.318 mmol) of PFA was dissolved together with 0.0167 g (0.06318 mmol) of MA-N-BP in 5 mL of TCTFE in a Schlenk tube and
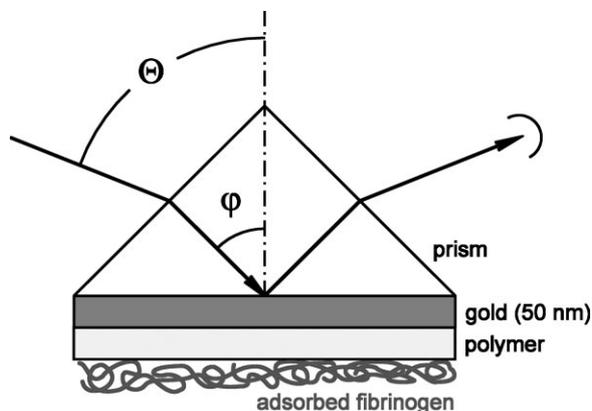
Figure 2. Schematic description of SPR set-up used for the protein adsorption experiments.

subjected to three freeze-pump-thaw cycles to remove dissolved oxygen. Subsequently, 8 mg (0.02 mmol) of AIBN was added and the polymerization reaction was carried out for 230 min at 60 °C. The highly viscous polymer was diluted by adding another 5 mL of TCTFE and precipitated into a ten-fold excess of methanol. The precipitate was filtered and vacuum dried overnight at 10^{-2} mbar pressure to remove volatiles. Around 3 g of polymer was obtained (yield = 92%). The amount of benzophenone containing monomer incorporated into the polymer chain was verified using proton-NMR measurements and was found to be 1 mol-% relative to the PFA monomer as expected.

Device Fabrication

The microfluidic platform used is of the same format as a conventional CD. The polymer disk features microstructures such as channels and reservoirs with minimum lateral dimension of 150 μm and depths of 40 μm in the shallow and 150 μm in the deeper areas. They are fabricated by standard hot embossing techniques using disks made from poly(ethylene-co-norbonene) COC (Topas 8007, Ticona GmbH) as substrates. A section of the microfabricated disk featuring various microstructures is depicted in Figure 1.[4–8]

Immobilization of Hydrophilic Polymers onto PMMA Layers for SPR Measurements

14 nm thick PMMA films were deposited on lanthanum slides which had been coated prior to film deposition with 50 nm gold films. The polymer was deposited by dip-coating from a 3 mg · mL^{-1} solution in THF at a typical withdrawal speed of 60 mm · min^{-1}. Subsequently a 15 nm thick PEtOx-BP film was formed on top of the PMMA film by dipcoating from a 2.5 mg · mL^{-1} solution of the polymer in dichloromethane at the same withdrawal speed. Film thicknesses were measured separately by SPR. After film deposition the samples were air dried and subjected to UV irradiation for 60 min using a Stratalinker® 2400 UV crosslinker (365 nm) using an energy density of 4.0 mW · cm^{-2}. Subsequent to illumination, the samples were extracted with water and dichloromethane to remove any non-attached polymer chains.

Planar structures are coated with the benzophenone containing copolymers in a conventional dip coating process. A local hydrophobic coating in the channel is achieved by dispensing 1 mg · mL^{-1} solution of the polymer in TCTFE either manually using a pipette or through an automated dispenser. After evaporation of the solvent the fluoropolymer is photocrosslinked and extracted. The microchannels on the disk are sealed by a thermal bonding process. A COC foil (Topas 8007, Ticona GmbH) featuring inlet and outlet holes is firstly aligned to the microfluidic structures and subsequently laminated on the substrate under a temperature of 90 °C and a pressure of 2.5 bar. Temperature and pressure are chosen such that a strong bond between both partners at a minimum bending of the lid into the channels due to thermal deflection is achieved. The setup of the lamination process is schematically depicted in Figure 4.

Figure 3. Optical image of microfluidic channels embossed into a CD consisting of polyethylene-*co*-norbornene.

Figure 5. Schematic description of the photocrosslinking reaction that occurs between the photoactive benzophenone groups within the polymer and the polymeric substrate upon irradiation.
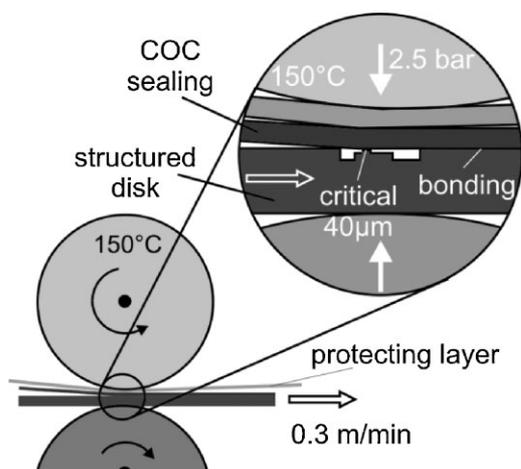

Figure 4. Schematic description of the set-up used for the lamination process.

Results and Discussion

To improve the surface properties of the microfluidic device polymers containing benzophenone groups are coated over the substrate to be modified either by spin or dip coating. Upon UV irradiation at 365 nm, photo crosslinking occurs between the photoactive benzophenone groups within the polymer and the polymeric substrate as shown in Figure 5. This layer remains stable even after successive extraction with solvents.

Various polymers were attached this way to the PMMA and COC surfaces without any problems. However, the deposition of very hydrophilic polymers such as PEtOx onto the COC was not quite as straightforward. When it was attempted to deposit relatively thin films of such a polymer from dilute water or alcohol solutions onto the COC surfaces serious wetting problems were encountered. The solutions did not wet the disk sufficiently to allow homogenous film
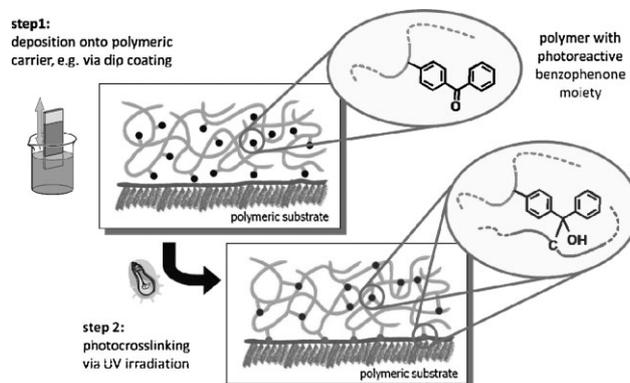
deposition and any attempts to coat the "bare" COC surfaces failed. One solution to the problem is to deposit a compatibilizing layer of a moderately hydrophobic polymer first. Then dewetting becomes a less serious issue and the desired hydrophilic coating can be applied in the second step. Alternatively, the COC substrate containing the microfluidic channels can be subjected to 10 min of water plasma to generate some (transient) hydrophilicity. Although this surface treatment does not have long-term stability it allows in a directly following step the application of 40 nm thick PEtOx coating using conventional dip coating techniques. The coated substrates were subsequently treated with UV light ($\lambda = 365$ nm) to attach the polymer to the channel surface.

The surface modification reduced the water contact angle from >80° for PMMA and COC substrates to 32° when PEtOx was used. This increased hydrophilicity allows now for the filling of the channels by capillary forces which does not happen with unmodified channels due to the hydrophobic nature of the substrate. The efficiency of the surface coating is directly evidenced in Figure 6, which shows a sample that was prepared as described above, but a mask was used to expose only one half of the microchannel to the UV
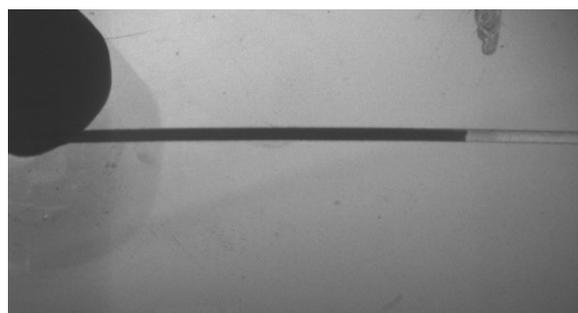

Figure 6. Optical image showing the wetting of only in the PEtOx modified areas of the microfluidic channel.

irradiation. The channel was then flushed with ethanol to remove any non-attached polymer. After drying of the disk a dye-colored buffer solution was placed in the injection port of the channel. As expected, the fluid fills the modified part of the channel easily by capillary forces and stops directly at the interface of the area, which was not exposed to the radiation.

Protein Adsorption onto Microchannel Surfaces

An important question for any bioanalytical application of microfluidic devices which needs to be addressed is how many of the biomolecules become attached to the surfaces of the microchannels. To allow for a quantitative evaluation of the situation at first model systems were studied by SPR measurements. A schematic description of the SPR device configuration is shown in Figure 2. For these experiments, gold-coated lanthanum glass slides were first covered with thin films of the same polymer used for the CD-fabrication[4–8] (i.e., PMMA or COC) in a simple dip-coating procedure. The slides were attached to the base of lanthanum glass prisms with the help of an appropriate index matching fluid. A flow cell was clamped to the slide and the whole assembly used for SPR measurements. Similar experiments were carried out using samples onto which thin layers of various polymers were deposited and photoattached. Although a variety of polymers were successfully tested, only PEtOx and PPFA films will be discussed in the following. The only prerequisite for the preparation of the samples was that the polymer which was to be photoattached did not dissolve the polymer film acting as the substrate (i.e., the PMMA or COC). Fibrinogen was chosen as a model protein, as it shows strong adhesion to many different surfaces, as it is a blood protein involved in blood clotting and wound healing.

A typical reflectivity curve obtained in the SPR experiment on a 14 nm thick PMMA film on a gold-coated lanthanum substrate is shown in Figure 7. This substrate is then coated with different polymers and the kinetics of protein adsorption to this surface is studied in situ. To this the SPR instrument was set to an angle approximately 0.5° below the angle of the minimum of the resonance signal and the intensity recorded as a function of time. The results of such in situ SPR measurements are shown in Figures 8a–d. After recording for some time the baseline the buffer solution initially present in the flow cell was replaced by the fibrinogen solution (0.01 M in PBS buffer). After the adsorption process had commenced for the chosen period of time the protein solution was again replaced by buffer solution. As the position of the minimum in the reflectivity curve which is due to the plasmon signal shifts as a dielectric protein layer becomes adsorbed to the surface, the reflectivity increases. The reflectivity of the surface in the
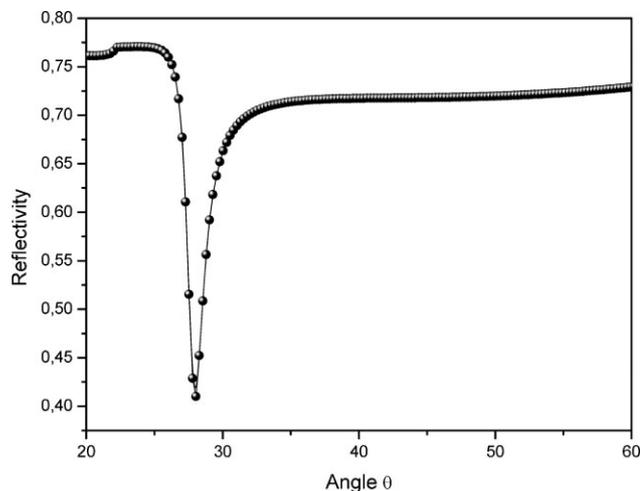

Figure 7. A typical reflectivity curve obtained in the SPR experiment on a 14 nm thick PMMA film on a gold coated lanthanum substrate (solid circles – experimental data; line – fit).

SPR experiment is thus a direct measure of the adsorbed amount of fibrinogen as long as the plasmon resonance signal shifts not too strongly (i.e., only so much that the slope of the reflectivity curve of the resonance signal remains almost linear). In Figure 8a it can be seen that upon the protein solution flowing in, a spontaneous, irreversible adsorption of fibrinogen occurs on unmodified PMMA surfaces. A layer of fibrinogen starts to accumulate at the surface of the substrate rather quickly. This layer can only to a small extent be washed off during subsequent washing cycles and approximately 8 nm of protein remain on the surface as determined by SPR angular scans after drying of the sample. Subsequently, the surface was modified with several different polymers through the photoimmobilization process described above and tested for protein adsorption. One of the candidates was a strongly fluorinated polymer as this gives strongly hydrophobic and even lipophobic surfaces. The deposition of a thin layer of the polymer indeed reduced the amount of protein adsorbing to the surface very significantly. However, it is also evident that a certain amount (~3–4 nm) of protein still adsorbed to the surface despite its low surface free energy. (Figure 8b)

In a second series of experiments poly(ethyloxazoline), a hydrophilic polymer, was attached to the surfaces of the polymeric substrates. To this, benzophenone containing copolymers of partially hydrolyzed poly(ethyloxazoline) were synthesized, attached to PMMA films and subsequently tested for protein adsorption. It can be easily seen in Figure 8c that the PEtOx coated surfaces did not adsorb any protein within the experimental error of this method and that such coatings are quite efficient in preventing the non-specific adsorption of the proteins to the substrate polymers surfaces.
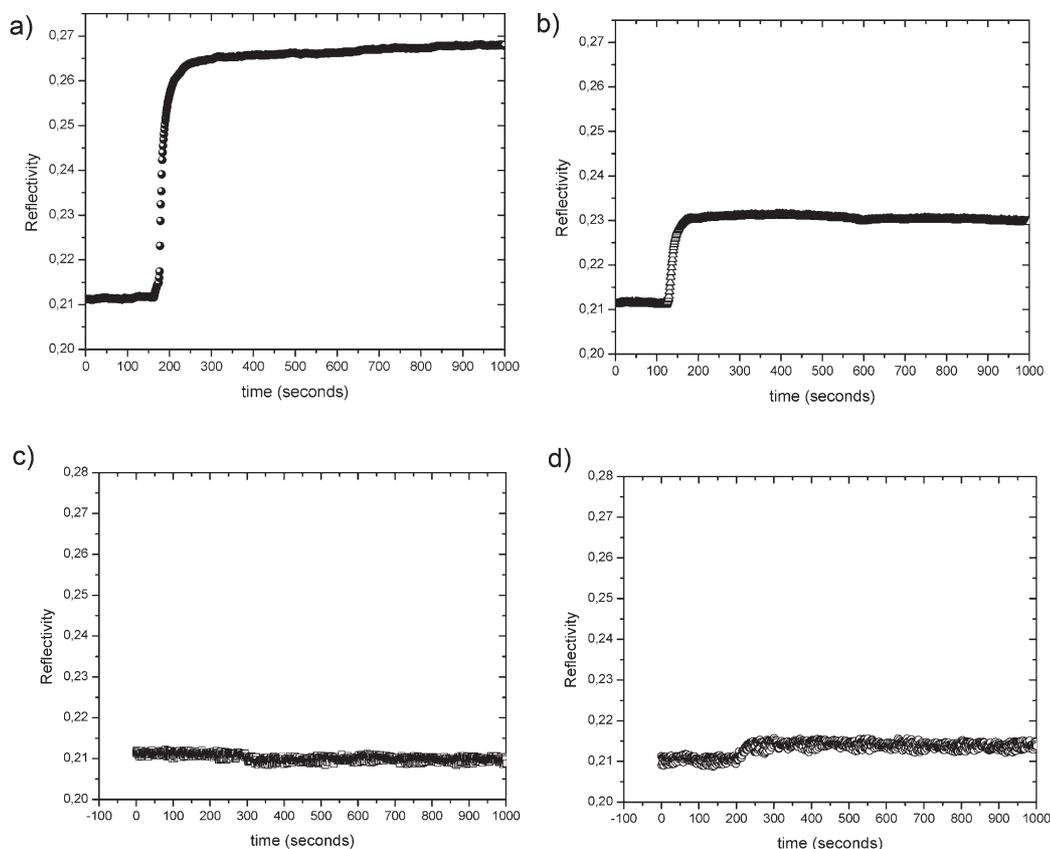
Figure 8. Measure of change in reflectivity with time in SPR experiments monitoring the adsorption of fibrinogen on (a) PMMA (b) PPFA (c) PEtOx (d) PEG-thiol.

To benchmark the results obtained, they were compared with self-assembled monolayers of polyethyleneglycol (PEG) with thiol end groups self-assembled on bare gold surfaces (Figure 8d). Polyethyleneglycol and its monolayers are known to reduce the protein adsorption of protein considerably.[46] This property of PEG layers is indeed seen in the SPR curves, where such surfaces show a strongly reduced protein adsorption behavior and a maximum of 2 nm of protein remain on the surface. In comparison, however, it appears that the hydrophilic polymers used in this study were even more efficient to prevent fibrinogen adsorption as no protein could be detected after washing and drying of the sample.

In order to prove that the surface modification process is not only working on planar model surfaces, but also on real microchannel systems,[4–8] a fluorescently labeled protein (Cy-5 fibrinogen) was flown through both modified and unmodified (sealed) COC channels. Fluorescence microscopy measurements (Figure 9a) show that the fluorescent protein solution completely fills the modified COC channel even though no additional pressure was applied. In contrast to this, in order to fill the unmodified channel a significant pressure had to be exerted. The effect of adding a hydrophobic spot can be seen directly in Figure 9b, where

a patch of the fluoropolymer PPFA (water contact angle on the patch: 120°) was deposited in the otherwise hydrophilic channel. If no additional hydrostatic pressure was applied the channel filled only partially until the liquid front reached the hydrophobic patch. However, of course if a pressure large enough is applied the hydrophobic patch can be overcome.

In Figure 9c a situation is shown where the solutions were drained from the unmodified channels, washed with buffer solution and fluorescence measurements were recorded. The channels still show a substantial fluorescence even after removal of the protein solution, indicating the presence of non-specifically bound proteins attached to the channel wall surfaces. In comparison to that in Figure 9d a, channel is shown, half of which was modified with a thin PEtOx film with a hydrophobic spot placed in the middle and filled with the fluorescent protein solution as shown in Figure 9b. After draining of the protein solution no significant irreversible fluorescence was observed in the areas modified with a thin PEtOx film, indicating that the protein was more or less completely removed.

In order to evidence the effect of the surface modification by the deposited PEtOx film more directly in Figure 9e a channel is shown where one half of it is subjected to 10 min
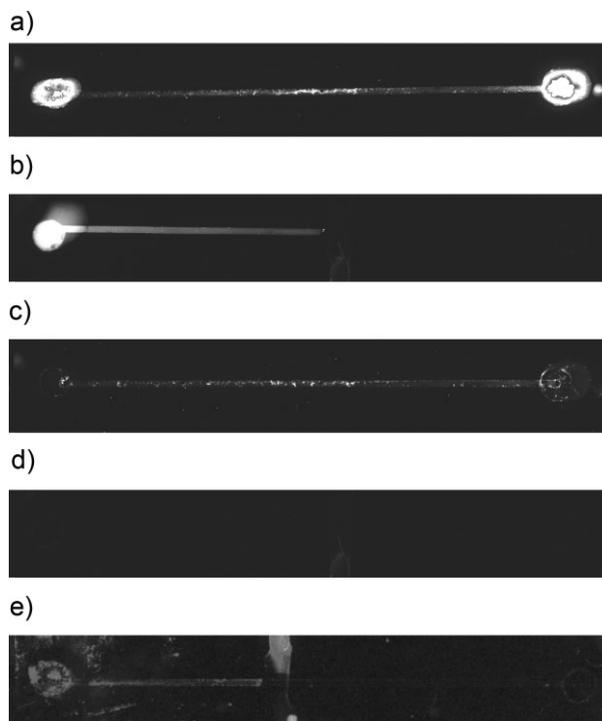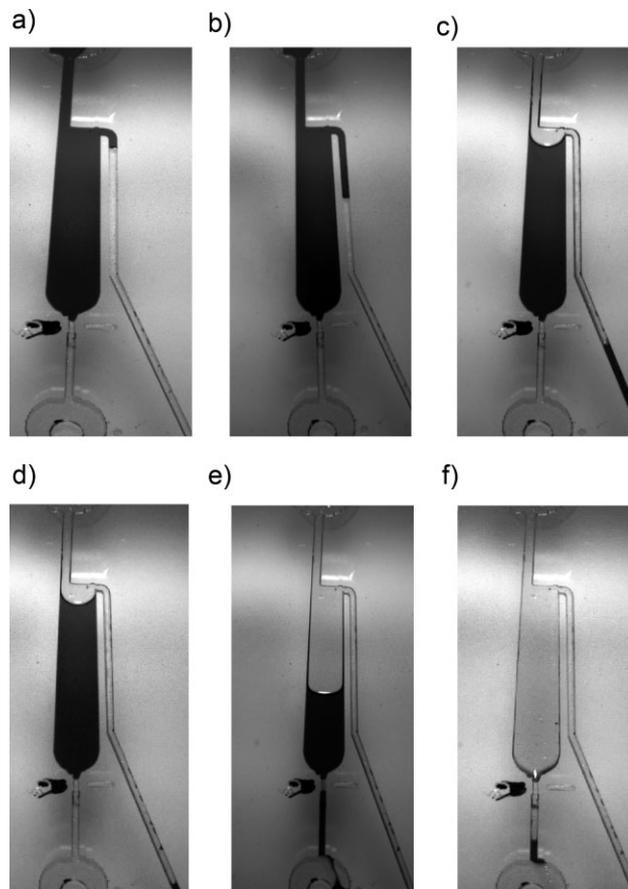
Figure 9. Fluorescence microscopy images of (a) Cy-5 labeled fibrinogen solution filled into a PEtOx modified microfluidic channel (b) Cy-5 labeled fibrinogen solution filled into a PEtOx modified microfluidic channel containing a hydrophobic patch in the middle (c) Unmodified microfluidic channel filled with Cy-5 labeled fibrinogen solution and subsequently drained and washed with buffer solutions (d) PEtOx modified microfluidic channel containing a hydrophobic patch in the middle after draining of Cy-5 labeled fibrinogen and rinsing with buffer (e) selective water plasma treated microfluidic channel filled and drained with Cy-5 labeled fibrinogen and washed with buffer.

Figure 10. (a) Filling of a reservoir and channel on a disk using a dyed water solution (b) – (d) overflow of excess solution; (e) – (f) draining of 10 nL of solution at a spin speed beyond the critical burst frequency of 20 Hz.

of water plasma, the other half remained unchanged. After filling the channel with protein solution, draining it and washing it with buffer solution, it can clearly be seen that one half of the channel wetted by the protein solution still showed significant non-specific protein adsorption in contrast to that observed in Figure 9d where the deposition of PEtOx subsequent to water plasma treatment completely prevented any significant protein adsorption. Further tests have shown that the PEtOx layers kept this property i.e., remained stable and protein repellent even when stored in PBS buffer for several weeks.

Hydrophobic Patches Inside Microfluidic Channels

The use of UV light to attach polymers onto surfaces of other polymers opens up the possibility to selectively pattern the micro channels and introduce hydrophobic patches inside of the hydrophilic channels. A small patch of a fluoropo-

lymer containing benzophenone as shown in Figure 1 was created on the microfluidic channel already covered with PEtOx. After UV irradiation the modified substrates were sealed by thermal bonding. The hydrophobic patch creates an abrupt change in the capillary pressure at a well-defined channel position. As the contact line cannot move any further it stops the capillary filling of the channel with the liquid. To overcome this barrier, an external pressure, for example generated by hydrostatic or centrifugal forces, is required that exceeds a certain critical value required for pushing the contact line across this barrier. The exact pressure required depends critically on the difference of the surface free energy of the two materials involved and on the exact geometry of the contact line.[4–8] A very interesting key feature of disk-like microfluidic devices is that the driving force pushing the liquid through the microfluidic channels is the centrifugal force. This force, however, can be easily controlled by simply altering the speed of rotation.

A sequence of images obtained during addition of 10 nL of dye-colored water flowing through such a surface modified channel while the disk is spinning is shown in Figure 10a–f. Water fills the structure by capillary and/or centrifugal forces in the areas made hydrophilic by the PEtOx (Figure 10a) and stops at the hydrophobically coated channel segments. The contact line comes to rest and does not proceed any further even if the disk is spun for prolonged periods of time at this frequency. If the rotational frequency of the disk is, however, raised above a critical burst frequency of 20 Hz, the centrifugal pressure on the liquid exceeds the capillary pressure, the liquid passes over the barrier and, as no additional liquid is supplied, the channel drains. This valving behavior can be used advantageously for the measurement and dispensing of a well-defined volume while the disk is rapidly spinning as shown in Figure 10. In the first phase of the dispensing process the disk is spun at a moderate speed, so that the liquid flown into the channel cannot cross the hydrophobic barrier and is dammed up behind it. As a consequence the whole volume behind the hydrophobic spot starts to fill up until a built-in overflow is reached (Figure 10a). Additional liquid added to the channel directly flows into the overflow channel and is removed (Figure 10b–c). If now the liquid supply is cut off the whole volume between the hydrophobic patch and the overflow is filled by 10 nL of liquid (Figure 10d). When the critical burst frequency of 20 Hz for this valve is exceeded the volume is drained and a precise volume of in this case 10 nL is ejected (Figure 10e,f).

Conclusion

Microfluidic channels generated in a rather hydrophobic polyolefin material have been successfully modified through the photochemical attachment of thin polymer networks to the surfaces of the channels. Although the present study just focused on two examples, this technique allows to use a wide spectrum of polymers including hydrophilic and hydrophobic (co-)polymers, which allows tight control of the surface chemistry of the surface chemistry of the devices.

The attachment of hydrophobic patches in the channels can be used for the generation of passive microfluidic valves, which allow the passage of liquids only if an external force beyond a certain threshold value is applied. Such valves permit the filling and draining of given volumes and thus a precise dosing, even if the device is in a non-stationary condition such as the rapidly rotating CDs employed in this study. In addition the photochemical surface coating process allows the generation of microfluidic devices with protein resistant surfaces i.e., surfaces which show no or very little unspecific adsorption which is an important feature for bioanalytical devices, especially if biomolecules having a very small concentration are to be detected and quantified.

As the surface attachment of the coating is light directed, precise spatial control of the surface chemistry of the channel surface can be achieved. The combination of precise fine-tuning of the surface chemistry and good spatial control opens avenues for the generation of tailor-made microfluidic devices.

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