# UNIVERSAL HYDROPHILIC COATING OF THERMOPLASTIC POLYMERS CURRENTLY USED IN MICROFLUIDICS

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**Abstract. A number of materials used to fabricate disposable microfluidic devices are hydrophobic in nature with water contact angles on their surface ranging from 80 to over 100 degrees. This characteristic makes them unsuitable for a number of microfluidic applications. Both the wettability and analyte adsorption parameters are highly dependent on the surface hydrophobicity. In this article, we propose a general method to coat the surface of five materials: polydimethylsiloxane (PDMS), cyclic olefin copolymer (COC), polyethylene terephthalate (PET), polycarbonate (PC), and polytetrafluoroethylene (PTFE). This fast, robust process, which is easily implementable in any laboratory including microfabrication clean room facilities, was devised by combining gas-phase and wet chemical modification processes. Two different coatings that improve the surface hydrophilicity were prepared via the "dip and rinse" approach by immersing the plasma oxidized materials into an aqueous solution of two different poly(dimethylacrylamide) copolymers incorporating a silane moiety and functionalized with either *N*-acryloyloxysuccinimide (NAS) (poly-(DMA-NAS-MAPS) or glycidyl methacrylate (GMA) (poly(DMA-GMA-MAPS). The coating formation was confirmed by contact angle (CA) analysis comparing the variation of CAs of uncoated and coated surfaces subjected to different aging treatments. The antifouling character of the polymer was demonstrated by fluorescence and interferometric detection of proteins adsorbed on the surafce. This method is of great interest in microfluidics due to its broad applicability to a number of materials with varying chemical compositions.**

## 1. Introduction

A number of thermoplastic polymers, such as cyclic olefin copolymer (COC), polyethylene terephthalate (PET), polycarbonate (PC) and polytetrafluoroethylene (PTFE), are currently used to fabricate disposable microfluidic devices via plastic machining technologies such as injection molding, casting, and embossing (1-3). The low cost and ease of both handling and manipulating these materials has stimulated their use in mass-produced polymeric lab-on-chip systems (4).

Another material widely used in microfluidics is polydimethylsiloxane (PDMS), a silicon-based organic polymer easily manufactured via soft lithography (5).

The hydrophobic nature of disposable plastic and PDMS devices presents a considerable drawback to microfluidics applications (6,7). For instance, the introduction of aqueous solutions into narrow channels is complicated by the low wettability of these materials. In addition, the hydrophobicity of many polymeric and inorganic materials makes them unsuitable for use with biological samples. It is generally recognized that proteins, which are attracted to hydrophobic and electrostatic

interactions, irreversibly adsorb onto the surface of plastic microdevices, which leads to poor analytical performance. Much effort has been devoted to modifying the surface of polymeric materials to adjust their wettability, adhesion and biocompatibility (8).

The various developed polymer surface modification techniques have been reported in several reviews (9-13). The most common modification methods fall into three categories: gas-phase processing, wet chemical methods and a combination of both. Gas-phase processing methods include plasma oxidation (14-16), ultraviolet (UV) irradiation (17-18) and chemical vapor deposition (CVD) (19). Wet chemical methods include layer-by-layer (LBL) deposition (20), sol–gel coatings (21), silanization (22), dynamic modification using surfactants (23) and protein adsorption. Finally, the combined gas-phase and wet chemical methods includes silanization and LBL methods on PDMS pretreated via methods such as plasma oxidation.

A general drawback to gas phase methods such as UV/ozone, corona, and various types of plasma treatments is the rapid aging of their surface (24). The plasma degrades a thin layer of the material and produces low molecular weight (Mw) oxidized polymer chains on the surface, which causes a hydrophilization that is rapidly lost in solvents due to the dissolution of low Mw chains [25]. Therefore, such treatments are not suitable for devices that contact liquids.

A similar instability problem is observed for PDMS treatments (26). In this case, there is a fast recovery of the hydrophobicity because of the migration of uncured PDMS oligomers from the bulk to the surface. In addition, mobile polymer chains containing Si-OH bonds tend to orient themselves toward the bulk at room temperature, which leads to dramatic surface changes over a short time.

Stable hydrophilic surfaces are produced on plastics using techniques that promote the formation of hydrophilic polymer coatings via either grafting- or plasma-induced polymerization methods (27-29). Using these methods on the production scale is generally complicated as they often require long treatment times and/or the handling of hazardous chemicals.

Surface modification through the layer by layer (LBL) deposition of polyanions and polycations is an emerging simple and efficient method for controlling the coating thickness on the nanoscale. Unfortunately, the functionality and stability of coatings obtained using this approach depend on many factors that are difficult to control, such as both the polyelectrolyte ionic strength and concentration, type of solvent, temperature, pH, etc. (30).

Another approach to improving the surface properties of PDMS is silanization, a process based on the condensation of silanol groups via the oxidation of functional alkoxy or chlorosilanes. While plasma-based oxidation is an established process, in situ wet chemical oxidation is less well characterized. A number or polymers bearing reactive functionalities have been grafted onto

silanized PDMS. For instance, O-[(N-succinimidyl)succinyl]-O-mPEG, poly(dimethylacrylamide-co-glycidyl methacrylate) (poly(DMA-GMA)), poly(vinylpyrrolidone)-g-glycidyl methacrylate (PVP-g-GMA) and poly(vinyl alcohol)-g-glycidyl methacrylate (PVA-g-GMA) have been covalently grafted onto APTES modified PDMS surfaces (31). These polymers are attached to the surface owing to the reactivity of surface functionalities to the chemically reactive polymer groups. Both these and other processes combining UV or plasma treatments with graft polymerization rely on the reproducibility of silanization which is known to be difficult to control even on glass, which is the most reactive material for organosilanes (32).

In this article, we investigate the surface properties of five materials including Sylgard R 184, a widely used and commercially available brand of PDMS, COC, PET, PC, and PTFE modified with a hydrophilic/antifouling coating characterized by high stability. A fast, robust process that is easily implementable in any laboratory, including microfabrication clean room facilities, was devised by combining gas-phase and wet chemical modification processes. Two different coatings were prepared via the "dip and rinse" approach by immersing the plasma-oxidized materials into an aqueous solution containing two different poly(dimethylacrylamide) based copolymers at room temperature before washing with water. As shown in Figure 1, polymers comprising a segment of poly(dimethylacrylamide) and incorporating a silane comonomer were functionalized with either *N*-acryloyloxysuccinimide (NAS) (poly-(DMA-NAS-MAPS) or glycidyl methacrylate (GMA) (poly(DMA-GMA-MAPS) comonomers. Pirri et al. used the copolymer containing NAS to bind biomolecules to glass slides for microarray technology (33). The coating procedure is easy, fast, robust and provides hydrophilic functional films covalently bound to the surface.

In this work, it was found that polymers bearing silane groups are effective at forming thin films on different thermoplastics as well as on PDMS. The presence of silane groups pending from the polymer backbone is critical for stabilizing the coating on a number of plastics. The other components in poly-(DMA-NAS-MAPS) and poly(DMA-GMA-MAPS) also play an important role in stabilizing the coating: DMA interacts with the substrates through both hydrogen and hydrophobic bonds whereas NAS or GMA react with the surface hydroxyl groups produced on the various plastics via oxygen plasma induced oxidation. Once the film forms, a simple reaction with an amino group (such as those present in ethanolamine or amino-PEG) converts the NAS into an unreactive moiety, which improves the antifouling properties of the coated surface without interfering with the stability of the attachment. In this work, we demonstrate that these two copolymers can stably coat a variety of materials with different chemical structure. It was also shown that, by modifying NAS with GMA the functionality of the surface polymer layer changes.

Unblocked NAS allows the surface to covalently bind proteins whereas GMA prevents such adhesion. The design of surfaces to which analytes specifically bind is important for biosensors and other technologies, e.g., affinity chromatography, coatings for implants, cell culture and artificial organs. Covalently attaching a protein to the biosensor is essential for its function whereas non-specific protein adsorption may compromise this performance. The coatings reported in this work bind proteins under certain conditions and resist the nonspecific adsorption of other proteins upon blocking. An extensive characterization of the hydrophilicity, binding capacity and resistance to unspecific protein adsorption of these coatings was performed using different materials. Contact angle measurements were obtained after subjecting the coating to harsh conditions to assess the irreversibility of the surface treatment.

## 2. Materials and methods

### 2.1 Chemicals

SYLGARD® 184 (PDMS elastomer kit) was purchased from Dow Corning (Midland, MI, USA). Ammonium persulphate ($(NH_4)_2SO_4$), sodium azide ($NaN_3$), phosphate buffered saline (PBS), ethanolamine ($NH_2EtOH$), sodium chloride (NaCl), Tween 20, N,N-dimethylacrylamide (DMA), glyicidyl methacrylate (GMA), [3-(methacryloyl-oxy)-propyl]trimethoxy-silane] (MAPS), allyl glycidyl ether (AGE), *N,N,N',N'*-tetramethylethylenediamine (TEMED) and Human Serum Albumine-Cyanine5 (HSA-Cy5) were purchased from Sigma Aldrich (St. Louis, MO, USA). All solvents were used as received. Oligonucleotides were synthesized for hybridization testing by MWG-BiotechAG (Ebevsberg, Germany) and contained the following sequences: COCU8: 5'-$NH_2$-GCCCACCTATAAGGTAAAAGTGA-3', COCU12: 5'-GCCCACCTATAAGGTAAAAGTGA-3' COCU10: 5'-Cy3-TCACTTTTACCTTATAGGTGGGC-3'. COCU10 was labeled with fluorophore Cyanine 3. All of these oligonucleotides were freeze-dried and re-suspended in DI water at a final concentration of 100 μM before use. *N*-acryloyloxysuccinimide was synthesized as reported elsewhere (34). The amorphous Cyclic olefin copolymers TOPAS 8007 and TOPAS 6013 were obtained for this study from TOPAS Advanced Polymers, Frankfurt-Höchst, Germany. TOPAS 8007 samples 1 mm thick, 25 mm wide, and 75 mm long were molded using an injection molding machine (Battenfeld HM 25/60). Polyethylene terephthalate (0.9 mm thick), polycarbonate (3.0 mm thick) were obtained from ArtaPlast AG, Rapperswil, Switzerland. Polytetrafluoroethylene was obtained from Lanza Nuova Spa Gandosso Bergamo Italy. Sylgard 184 was obtained from Dow Corning Corporation. Glass microscope slides were dip coated with a 2-3 mm thick PDMS

layer. The elastomer was mixed with the curing agent in a 10:1 ratio. A glass slide was immersed in the mixture and cured for 4 h at 80°C. No washing step was performed.

### 2.2. Processes

***Synthesis of poly-(DMA-NAS-MAPS) and poly-(DMA-GMA-MAPS).*** Poly-(DMA-NAS-MAPS) and poly-(DMA-GMA-MAPS) were both previously synthesized and characterized (35). Briefly, the polymers were synthesized via a random radical polymerization in anhydrous tetrahydrofuran with a 20% w/v total monomer concentration. The DMA, NAS and MAPS monomer molar fractions were 97:2:1, whereas the DMA, GMA and MAPS molar fractions were 95:4:1. In a 100 mL three-neck round-bottom flask, 20 mL of anhydrous THF was degassed under vacuum for 20 minutes. DMA, (filtered on aluminum oxide to remove the inhibitor), GMA or NAS and AIBN (0.01 g, 0.08 mmol) were added under nitrogen, and the stirred solution was degassed for an additional 10 minutes under vacuum. MAPS was subsequently added under nitrogen, and the solution was polymerized at 65°C for 2 hours. The reaction was stopped by cooling to room temperature; the polymer solution was diluted 1:1 with anhydrous THF and precipitated in 400 mL of petroleum ether. The product was collected as a white powder after filtering with a Buckner funnel and drying under vacuum at room temperature.

***Plastic surface coating.*** The oxidized plastic surfaces were produced via the oxygen plasma treatment of the slides in a Plasma Cleaner from Harrick Plasma (Ithaca, NY, USA). The oxygen pressure was set to 1.2 bar with a power of 29.6 W for 10 min. Immediately after oxygen plasma treatment, the ox-slides were transferred to solutions of poly-(DMA-NAS-MAPS) and poly-(DMA-GMA-MAPS) dissolved in DI water to a final concentration of 2 % w/v and then diluted 1:1 with an aqueous $(NH_4)_2SO_4$ solution at 40% of saturation. The slides were immersed into the respective coating solution for 30 minutes, rinsed in DI water, dried with nitrogen flow and then cured in a vacuum at 80°C for 15 minutes.

***Goniometry.*** Contact angle measurements were collected via the sessile drop method using a CAM200 instrument (KSV Ltd), which utilizes video capture and subsequent image analysis. Deionized water was used, and its purity was confirmed by correlating the measured surface tension based on the pendant drop shape to the literature values for pure water (72 mN/m at 25°C).

***Coating stability.***

***Coating aging study.*** The DI water contact angles of both uncoated, oxygen plasma treated and coated surfaces were measured immediately after oxidation, coating and 10 days of storage at room temperature in a dessicator.

***Stability to solvent.*** The contact angles of freshly coated slides immersed in a EtOH 70 % (v/v) water solution for 2 minutes at room temperature were measured after rinsing with DI water and drying under a stream of nitrogen.

***Stability to high temperature.*** Fresh poly-(DMA-GMA-MAPS) coated slides were immersed in boiling water for 5 minutes, rinsed in DI water at room temperature and then dried under a stream of nitrogen. PET and TOPAS 8007 were immersed in water at 75°-80°C to avoid changes in their plastic morphology.

***Protein adsorption study.*** Multiwell cell culture system plates (see Figure 1S) were used to form wells on plastic slides. The uncoated plastic slides and multiwell frames were bound together at room temperature after oxygen plasma treatment by pressing the surfaces together using gentle finger pressure. The slides bonded to the frame were coated separately using the two polymer solutions prepared above. The oxidized slides coated with poly(DMA-NAS-MAPS) were first blocked with ethanolamine, while both the poly(DMA-GMA-MAPS) coated and uncoated reference slides were directly incubated with the protein solution. Each well was filled with 20 μL of a s1 μg/ml PBS solution of human serum albumin labeled with a cyanine 5 dye (HSA-Cy5) and left for 2 hours at room temperature. The slides were then washed with a washing buffer for 10 minutes, rinsed in water and dried under a stream of nitrogen. Finally the frames were removed and adsorbed protein was quantified using a ScanArray Lite confocal laser scanner from Perkin Elmer and analyzed using ScanArray Express software.

***DPI instrumentation and adsorption experiments.*** Dual polarization interferometry (DPI) measurements were conducted using an Analight Bio 200 (Farfield Group, Manchester, UK) running Analight Explorer software. The theory behind this technique, which measures optical phase changes in an evanescent dual polarization interferometer, ~~is described in detail elsewhere~~ (36). ~~Briefly, a laser beam used as the light source can be switched between two plane polarized states, the transverse electric (TE) and transverse magnetic (TM), via a fast liquid crystal switch. This light is focused on the short end of the DPI sensor, which consists of two horizontally stacked waveguides made of silicon dioxide doped with silicon nitride. An insulating layer separates the reference waveguide from the sensing waveguide, which is in contact with the solution of interest. The light beam from the laser is split and allowed to travel separately through these two waveguides. The light beams exiting the waveguides interfere with each other and thus create an interference pattern in the far-field. This interference pattern is captured by a CCD camera and recorded with a computer after A/D conversion. When polymer/protein molecules adsorb onto the sensing wave guide, the light traveling through the wave guide undergoes a phase shift, which~~

~~changes the interference pattern and provides information on the adsorbed layer morphology and adsorption kinetics.~~ These experiments were performed at 20°C using PBS or 50 mM Tris (HCl) running buffers at a pH of 7.6 and containing 150 mM NaCl, 0.02 % (v/v) Tween 20, and 1% bovine serum albumin (incubation buffer). A silicon oxynitride AnaChipTM surface treated with oxygen plasma was used in this study. To measure the coating thickness, the chip was inserted into the fluidic compartment of an Analight Bio 200 and treated with polymer solutions at 1% w/v in a 20% saturated ammonium sulfate solution that were slowly introduced to the chip channels at a flow rate of 6 µL/min for 15 minute. The flow was then stopped, and the solution was let in contact with the surface for 30 minute before washing the channel with PBS, which was injected into the channel at a flow rate of 50 µl/min.

The amount of protein adsorbed onto the polymer coated sensor surface was evaluated in a separate experiment; the sensor was coated off-line because the surface had to be dried off-line. Planar Anachips compatible with DPI experiments were coated with poly(DMA-NAS-MAPS) and poly(DMA-GMA-MAPS) by immersing the slides in a 1 % (w/v) polymer solution in ammonium sulfate followed by rinsing with water and drying under vacuum at 80°C. The active succinimide ester groups in NAS were blocked by incubating the coated chips with 50 mM ethanolamine in 0.1 M Tris (HCl) with a pH of 9. The coated sensor chips assembled within the instrument were flushed with a 10% v/v solution of bovine serum in the incubation buffer. Five serum injections of 40 minutes each were performed at a flow rate of 5µl/mL and separated by a 20 minutes PBS rinse. Before each experiment, a standard calibration procedure was performed using 80 % (w/v) ethanol and MQ $H_2O$ solutions. The data were analyzed using Analight Explorer software to calculate the mass of both the polymer and unspecific adsorbed serum proteins.

***Surface DNA immobilization.***

***Oligonucleotides immobilization.*** The oligonucleotides COCU8 and COCU12 were dissolved in a 150 mM sodium phosphate buffer solution at pH 8.5 with a final concentration of 10 µM. The oligonucleotides were spotted using a SCENION sci-FLEXARRAYER S5 non-contact microarray spotter with an 80 µm nozzle. The spot volume, temperature and humidity were precisely controlled to 400 pL, 22°C and 50%, respectively. One array was created in the center of each coated slide. An aqueous blocking solution containing 0.1 M TRIS/HCl buffer with a pH of 9 and containing 50 mM ethanolamine was heated to 50°C. The slides were immersed for 30 minutes and kept at 50°C before rinsing with DI water. A second solution containing 4X SSC and 0.1% SDS was prepared and pre-heated to 50°C. The slides were immersed for 15 minutes and kept at 50°C before rinsing with DI water and drying with a nitrogen flow.

***Hybridization.*** The spotted and blocked slides were incubated with a complementary oligonucleotide target COCU10 labeled with cyanine 3 for fluorescence detection. COCU10 was dissolved in a water solution containing 2X SSC, 0.1% SDS and 0.2 mg/ml of BSA with a final concentration of 1 μM. Next, 25 μl of this solution was deposited on the array, and a coverslip was used to cover the hybridizing area. The hybridization reaction was conducted in a humid chamber at 65°C for 2 hours. Finally, any unbounded oligonucleotides were removed using two 5 minute washes with an aqueous 4X SSC solution pre-heated to 65°C, a 1 minute wash with 0.2X SSC, and a 1 minute wash with 0.1X SSC followed by centrifugation to dry the slides. Images of each slide were obtained using the ScanArray Lite confocal laser scanner by Perkin Elmer and analyzed using ScanArray Express software.

***Statistical Analysis.*** For the statistical analysis, t-test was used to compare means for two groups of contact angle data obtained on slides of the same material subjected to different treatments (aging, solvent and temperature). The same test was also used to compare means for the two groups of fluorescence data obtained by incubating uncoated or coated surfaces with a fluorescent protein. A value of P (probability) < 0.05 was considered to indicate statistical significance. Statistical analyses were performed using Excel program.

## 3. Results and discussion

### 3.1. *Polymer coatings*

A number of thermoplastics and thermosets, widely used to construct microfluidic devices, have been subjected to a robust and fast coating treatment easily applicable in any laboratory. The materials considered, polydimethylsiloxane (PDMS), polycarbonate (PC), cyclic olefin copolymer (COC), and polyethylene terephthalate (PET), are broadly applicable to the fabrication of microfluidic devices. This study also includes a hydrophobic material, polytetrafluoroethylene (PTFE), which uses the trade name Teflon, whose surface is extremely difficult to modify. Polymeric films can adhere to a surface due to a combination of van der Waals, electrostatic and covalent interactions between the two materials. However, the interfacial adhesion between a polymer film and an oxidized material can be improved using a broad class of silane coupling agents. The general formula of a silane coupling compound used for improving adhesion is $(R'O)_3$-Si-R, where R´O- is an alkoxy group and –R is an organofunctional group. Under appropriate reaction conditions, the alkoxy groups condense with the hydroxyl groups available on the surface to produce a surface decorated with organofunctional –R groups, generally vinyl groups, which promote the formation of covalent bonds between the coupling agent and polymeric network. In

general, this process is conducted over two steps, first silanization and second polymerization, with the incorporation of vinyl groups to promote surface attachment. The approach we suggest herein is different because it incorporates MAPS into a polydimethylacrylamide backbone via copolymerization of the methacrylate moiety with dimethylacrylamide, which promotes the polymer adhesion in a single step. The trimethoxysilane goups pending from the backbone hydrolyze into silanetriol groups during adsorption in water. Silanols can condense with either hydroxyl groups generated via oxygen plasma oxidation on the surface or silanols belonging to other polymer chains, which forms a network. The success of this approach is likely due to the amphiphilic character of DMA, the backbone monomer, which interacts strongly with the surface through a variety of mechanisms, including hydrophobic interactions, van der Waals forces and hydrogen bonds. The presence of additional chemically reactive monomers (NAS or GMA) further stabilize the thin film on the surface in addition to conferring the ability to bind biomolecules to the coating.

### *3.2. Control of surface hydrophilicity*

The contact angle was measured both before and immediately after the coating deposition to monitor and quantify changes to the surface hydrophilicity resulting from the presence of a surface polymer layer. All materials in this study have water contact angles ranging from 100° to 80°. These values might render their use in microfluidics and medicine problematic. The surfaces of all samples, except Teflon, became more hydrophilic upon the oxygen plasma treatment. Figure 2 illustrates plots of the contact angles for the untreated, oxygen plasma treated and polymer-coated materials. The error bar has been computed based on the results from the repeated measurements at five different observation points on two slide replicates. The formation of a polymer coating is immediately evident for PDMS. For the other materials, even though the water droplet contact angles decreased to approximately 40° for both the poly(DMA-NAS-MAPS) and poly(DMA-GMA-MAPS) coatings, it was impossible to distinguish the effects of plasma treatment from those induced by the coating because the contact angles observed under both circumstances were not statistically different. However, when stored in air or washed with ethanol, the contact angles of PDMS and the thermoplastics increased, though to varying extents, whereas those of the polymer coatings remained almost constant. The non statistically relevant change of the CA values before and after exposure to air and ethanol was confirmed by a t test with $p$ (probability) lower than 0.05 (Figure 2S and 3S in Supplementary information). This finding is not surprising because the aging of plasma-treated surfaces is a well-known phenomenon with a rate dependent on the characteristics

of the material, plasma and surrounding environment. Activated surfaces may have a shelf-life of hours or days (37). An additional proof of the stability is given by the low contact angle variation observed in poly(DMA-GMA-MAPS) coated substrates after immersing for 5 minutes in boiling water (Figure3). Also in this case no statistical variation of contact angles was observed (t values from 1.3 to 4.5, *p* lower than 0.05. This test strongly suggests the presence of covalent bonds between the polymer and surface because a physically adsorbed coating would easily detach under these conditions. We attribute this stability to the silane monomers pending from the polymer backbone because in the absence of MAPS the contact angle increased markedly upon treatment with boiling water and reached values close to those of the uncoated substrates (data not shown) .

### *3.3. Control of protein adsorption*

Protein adsorption at solid–liquid interfaces is a process centrally important to biomedical technologies such as biosensors and biochips (38), biomaterials for medical implants (39, 40) and non-fouling surfaces. The accuracy and reliability of a biosensor, for instance, depends on both the ability of its surface coating to capture proteins in high density and its ability to prevent the unwanted adhesion of other proteins present in the matrix containing the analyte to be sensed. Non-specific adsorption of proteins plays a major role in reducing assay sensitivity and causes low signal-to-noise ratios. The selective/non selective binding characteristic of the surface is one of the reasons plastic microarrays have not been successful despite the ease of adapting plastic substrates to microfluidic systems. There is thus a need to develop polymeric materials with a high hybridization signal-to-background ratio to enable the sensitive detection of proteins. A large number of techniques based on various principles such as optical absorption, refractive index changes, radiolabeling, electromechanical microbalances, and fluorescence markers (see (41) and references therein) have been used to provide information on protein adsorption.

In this work, we demonstrate a reduction in the protein adsorption to poly(DMA-GMA-NAS) and poly(DMA-NAS-MAPS) coatings, the latter blocked with ethanolamine, using fluorescence and dual polarization interferometry (DPI), which is an optical surface analytical technique that provides multiparametric measurements of molecules on a surface to give information on the molecular dimension (layer thickness), packing (layer refractive index, density) and surface loading (mass) (42).

In experiments measuring the protein adsorption by fluorescence, both uncoated and coated PDMS and COC (TOPAS 6013) slides were incubated with a 1 mg/mL solution of Cy5 labeled human albumin. A removable PDMS frame was used to create eight wells on the slide and confine the proteins to specific zones on the surface. This confinement facilitates the quantification of fluorescence using a scanner. In Figure 4, the fluorescent intensity accounts for the amount of human albumin adsorbed on those areas exposed to the protein. The difference between the bare and coated materials is striking as shown by the t-test statistical analysis (PDMS slides: $P<0.05$, $t=8.983$, COC slides: $P<0.05$, $t=5.908$).

During the experiments to characterize the polymer coating using DPI, the surface of the sensor chip was coated with flowing poly(DMA-GMA-NAS) and poly(DMA-NAS-MAPS) as reported in section 2.2. To assess protein adsorption, five injections of 10% w/v bovine serum in the incubation buffer were performed using chips coated off-line as described in section 2.2, and the amount of protein adsorbed onto the polymer coated surface was assessed using the Farfield AnaLight Explorer software. The technology behind Analight Bio 200 is dual polarization interferometry, a label-free technique that measures the layer thickness with picometer resolution. Tables 1 and 2 show the mass, thickness, density and refractive index of both the polymer films and adsorbed serum proteins. The mass of adsorbed proteins in the coated channels is significantly less than that in the non-coated ones, which demonstrates the antifouling properties of the polymers. In particular, the mass for poly(DMA-GMA-MAPS) is below the limit of detection for the instrument. These experiments provide both a complete characterization of the polymer layers and an absolute quantification of the amount of protein nonspecifically adsorbed to the surfaces. The antifouling character of the polymer film, demonstrated on silicon nitrate, might be useful to reduce non specific adsorption of proteins also on other materials coated by he same polymer. The availability of high quality coatings enables the use of PDMS and plastics in a variety of bioanalytical microdevices that require minimal nonspecific adsorption of biomolecules.

### *3.4. Surface functionalization.*

The polymeric coating formed by poly(DMA-NAS-MAPS) on the surface of the various materials contains active esters that react with nucleophilic molecules under mild conditions. The reaction between the amino group of an amino-modified oligonucleotide and the succynimidyl ester on the polymer allows for the covalent immobilization of oligonucleotides on the surface. To exemplify the binding capability of surfaces coated with this polymer, coated COC (TOPAS 6013) was

spotted with picoliter volumes of a 22-mer aminomodified oligonucleotide in a microarray format as shown in Figure 5. The covalent attachment was demonstrated by measuring the fluorescence signal produced via the hybridization of an oligonucleotide complementary to those attached to the surface and labeled with Cyanine 3 dye. An oligonucleotide with the same sequence but lacking the amino terminus was spotted in the central part of the array to demonstrate that the binding is mediated by the covalent reaction between the complementary functional groups on the surface and the polymer and not the result of an unspecific adsorption. Oligonucleotides were chosen to prove the functionality of the surface because of their low tendency to adsorb on hydrophobic surface and high hydrophilicity. If protein probes were used, the polymer layer would have also bind them. However, it would be more difficult to demonstrate that the protein binding was mediated by the covalent reactions with the polymer layer as proteins also adsorb to uncoated surfaces. The complete absence of binding of the amino modified oligonucleotides to uncoated surfaces clearly demonstrates that a functional coating is formed and is responsible for the attachment of the oligonucleotide to the surface.

## 4. Conclusions

This work demonstrates that two copolymers, poly-(DMA-NAS-MAPS) and poly(DMA-GMA-MAPS), form a coating on the surface of various plastics including PDMS, COC, PET, PC, and PTFE. The coating is formed via a simple and robust dip and rinse treatment at room temperature and does not require the use of organic solvents. The marked hydrophilic/antifouling characteristics of the plastic surfaces modified with these copolymers was demonstrated through contact angles measurements and protein surface interaction quantification. The coating was found to be stable over time and survive both organic solvents and high temperature treatments. One of these two polymers, poly-(DMA-NAS-MAPS), conferred the ability to covalently bind biomolecules to the surface and thus paved the way for applying such coatings to microarrays.

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**Figure caption**

**Figure 1.** Chemical structure of the two polymers poly-(DMA-NAS-MAPS), poly-(DMA-GMA-MAPS) used to coat the plastic surfaces. Theoretical values of *n*, *m* and *p* in the monomer feed are 97%, 2% e 1%, respectively.

**Figure 2.** Plots of the water contact angles of untreated, oxygen plasma treated and polymer coated surfaces. The mean values of contact angles on coated and uncoated slides where found to be significantly different as confirmed by a t-test with P (probability) < 0.05.

**Figure 3.** Plots of water contact angles of poly-(DMA-GMA-MAPS) coated surfaces washed with boiling water. A statistical analysis, t-test with P (probability) < 0.05 as statistical significance, confirmed that the contact angles didn't change significantly in boiling water.

**Figure 4.** Fluorescent intensity of the surface area exposed to HAS-Cy5. The fluorescence accounts for the amount of HAS-Cy5 adsorbed to the surface. A statistical analysis, t-test with P (probability) < 0.05 as statistical significance, confirmed the difference in the amount of fluorescence detected on coated and uncoated surfaces .

**Figure 5.** Oligonucleotide hybridization experiment on the COC (Topas 6013) surface coated with poly-(DMA-NAS-MAPS). a) microarray scan showing the Cy3 fluorescence signal for the hybridization of the spotted oligos with COCU10, b) microarray spotting scheme.