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Microarray Glass Slides Coated with Block Copolymer Brushes Obtained by Reversible Addition Chain-Transfer Polymerization

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The reversible addition−fragmentation chain-transfer polymerization was used to prepare microarray slides grafted with polymer brushes for DNA-based applications. Block copolymer brushes of *N*,*N*-dimethylacrylamide (DMA) and glycidyl methacrylate (GMA), poly(DMA-*b*-GMA) were prepared by extending living poly(dimethylacrylamide) chains. The functional surface was used as a substrate for oligonucleotide hybridization experiments. The results were compared to those provided by glass slides coated by a self-assembled monolayer made of (3-glycidyloxypropyl)trimethoxysilane. Surfaces coated with block polymer brushes bearing oxirane groups are more efficient as substrates for oligonucleotide hybridization than surfaces coated with nonpolymeric self-assembled monolayers containing the same functional group. The high probe grafting density and hybridization efficiency achieved with this polymeric coating reveal the importance of the block architecture to ensure good accessibility of the immobilized probe. The new surface was characterized by static angle measurements and diffuse reflectance FT-IR spectroscopy on a silica model system.

The development of reactive surfaces and their use as substrates for oligonucleotides (ODNs), DNA, and protein microarrays has had a great acceleration in the past decade.[1] Microarrays consist of regularly repeating arrays of DNA or protein spots. The availability of functional surfaces, able to efficiently bind biomolecules, is mandatory to achieve specific discrimination in molecular recognition events that take place at the surface/solution interface.[2] The amount of biological material present within each spot determines the levels of sensitivity of microarray technology as well as its dynamic range and analysis speed. However, the parameter that ultimately affects mixed-phase hybridization efficiencies is not the total amount of probe immobilized within each spot but its grafting density. It is important to avoid steric crowding that can hinder hybrid formation.[3] Different types of surfaces have already been explored for arraying biomolecules, but the search for new supports giving superior characteristics is still a challenge.

Organosilane-based self-assembled monolayers (SAM) bearing different reactive groups[4−7] have been widely used in the field of microarrays due to the availability of a large variety of reagents and well-established coating procedures. However, it is experimentally found that, with this type of coating, the hybridization efficiency for an oligonucleotide target to its complementary probe is significantly lower than that expected due to the low accessibility of the immobilized probe, which lies flat on the surface.[8,9]

With respect to the problem of probe accessibility, three-dimensional coatings are advantageous in that they leave the immobilized biomolecules free to interact with their counterpart in solution. Different strategies have been proposed to realize three-dimensional surfaces. Macroporous silicon has been recently introduced as a substrate for highly sensitive protein chip applications. The formation of 3D porous silicon structures was performed by electrochemical dissolution of monocrystalline silicon.[10] In another recent article, a three-dimensional, uniformly porous substrate featuring a regular array of discrete, ordered, high aspect ratio microchannels has been described.[11] Systems of the type reported above, although very effective in creating 3D surfaces, require the use of technologies that are not suitable for large-scale application of the microarray support at low cost.

A simple way to create an aqueous three-dimensional environment on a surface is by depositing a polymeric coating of thicknesses ranging from a few nanometers to a few micrometers. Thin films of cross-linked polyacrylamide gels,[12−15] agarose,[16,17]

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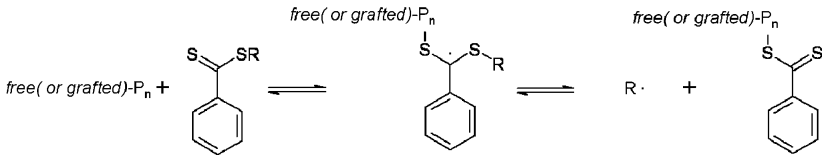
Scheme 1. Chain-Transfer Mechanism Responsible for RAFT Polymerization



electropolymerized polypyrrole,[18] and dendritic polymers[19,20] have been proposed to immobilize biological molecules with a density per surface unit ranging from 10^{12} to 10^{15} molecules/cm^2 depending on the characteristics of the polymer.[1]

Macromolecular chains tethered in solution, linked to the solid surface by one end, commonly named "brushes", provide a system in which the reactive groups are segregated at the end of the chain and spaced from the substrate by a long spacer.[21] To produce tethered chains on the surface, two different approaches are commonly employed: physisorption or covalent attachment of linear chains.[22] PEG-based polymer brushes, introduced by forming a self-assembled monolayer, are widely used in many biological applications.[23−26] An alternative approach to attach PEG polymers to a substrate involves living polymerization of PEG-containing acrylate monomers by a variety of mechanisms such as ATRP[27] or nitroxide-mediated CRP.[28] However, PEG brushes usually bear only one reactive group per chain. To increase the density of reactive groups, the combination of surface-initiated polymerizations and controlled polymerization techniques has been explored.

In this paper, we provide the first report on the successful immobilization of an oligonucleotide on a microarray glass slide coated with block copolymer brushes obtained by a *grafting from* approach, based on a radical addition−fragmentation transfer (RAFT) polymerization. RAFT is a controlled/living polymerization process used to synthesize well-defined polymers with low polydispersity index (I_d).[29] In this approach, polymer chains grow directly from an initiator immobilized on the surface leading to formation of chains covalently attached, by one end, to the substrate. RAFT has been widely used with a variety of monomers including acrylamide, and N- and N,N-disubstituted acrylamides, in both organic and aqueous media,[30−35] as a robust and industry friendly route to produce living homopolymers block and star polymers.[36−38] The process involves a conventional free radical polymerization in the presence of thiocarbonylthio compounds of general structure S=C(Z)SR, which convert chain-propagating radicals into a "dormant" form in equilibrium with the "active" form (Scheme 1). This category of molecules has the role of chain-transfer agent (CTA), being able to control the polymer growing process. In the literature, there are surprisingly few reports on the application of RAFT techniques[39−43] to the synthesis of polymer brushes, probably due to the difficulty of preparing RAFT agent anchored substrates.[44] The aim of this work was to develop a user-friendly method to initiate, from the glass surface, living polymer segments that terminate with a dithiobenzoic group (CTA). The CTA at the end of the chain allows the initiation of a second polymer segment comprising a reactive monomer. The confinement of reactive groups on the external portion of the polymer is a considerable advantage as it allows the binding of low or high molecular mass molecules with a conformation that resembles the one they have in free solution, thus facilitating the hybridization reaction with their biological counterpart.

The proposed brush block coating was compared with a SAM containing a 3-glycidyloxypropyl residue, the group that constitutes the functional block of the polymer. Coated glass slides were characterized by tensiometry and by diffuse reflection FT-IR (DRIFT) spectroscopy.[45] The analysis by DRIFTS, carried out on

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a silica model system, provided information on the film composition whereas tensiometry allowed us to monitor contact angle variations resulting from the growth of different polymer chains. In addition, a functional test based on an ODN hybridization experiment confirmed that the proposed method leads to an effective functionalization of the surface with at least 0.3×10^{13} accessible ODN molecules immobilized per square centimeter.

EXPERIMENTAL SECTION

Materials. Glycidyl methacrylate (GMA), *N*,*N*-dimethylacrylamide (DMA), (3-mercaptopropyl)trimethoxysilane (γ-MPS), (3-glycidyloxypropyl)trimethoxysilane (γ-GPS), benzoic acid, phosphorus pentasulfide, cyclohexane and petroleum benzine were supplied from Aldrich (Stanheim, Germany). GMA and DMA were purified on a neutral alumina column in order to remove the stabilizer. Sodium dodecyl sulfate (SDS), ammonium sulfate, tris(hydroxymethyl)aminomethane (TRIS), tetrahydrofuran (THF), ethanolamine, bovine serum bovine (BSA), and 20× SSC (3.0 M sodium chloride, 0.3 M sodium citrate) were supplied from Sigma (St. Louis, MO). Sodium hydroxide and chloridric acid were supplied from Serva (Heidelberg, Germany). *N*,*N*-Dimethylformamide (DMF), toluene, ethanol, potassium bromide (spectroscopic grade), and α,α′-azoisobutyronitrile (AIBN) were from Merck (Darmstadt, Germany). Silica (Davisil 663XWP, 500-Å pore size, 78−85 m^2/g surface area) was obtained by Supelco (Bellefonte, PA).

Synthesis of Cyanoisopropyl Dithiobenzoate.[46] Benzoic acid (600 mg, 5.13 mmol) was dissolved in dry and degassed toluene. P_4S_{10} (550 mg, 1.2 mmol) and AIBN (2.5 g, 15 mmol) were added to this solution, under stirring and nitrogen. The reaction flask was warmed at 110 °C for 1 h. After cooling, the solution was poured into cyclohexane and then filtered. A crude sample, containing the cyanoisopropyl dithiobenzoate (R_f 0.8 in $CHCl_3$), was recovered and purified by flash chromatography first on silica (CH_2Cl_2) and then on neutral alumina (petroleum benzine/CH_2Cl_2 9/1) with an overall yield of 40%. ^{1}H NMR (δ ppm, $CDCl_3$): 7.93 (d, 2H, *o*-ArH), 7.56 (dd, 1H, *p*-ArH), 7.4 (dd, 2H, *m*-ArH), 1.95 (s, 6H, 2 CH_3).

Microscope Slide Functionalization. *Slide Pretreatment.* Glass slides were immersed in EtOH for 1 h, washed with distilled water, dried, and dipped in 1 M NaOH solution for 30 min. After these treatments, the slides were immersed in 1 M HCl for 30 min, washed with water to neutrality, and dried in an oven at 80 °C.

Organosilanization with (3-Mercaptopropyl)trimethoxysilane. The slides were covered with a 10% v/v (3-mercaptopropyl)-trimethoxysilane solution in dry THF and left in this solution for 12 h at room temperature under nitrogen. After the reaction was completed, the slides were washed with THF and with acetone, and then dried under nitrogen in oven at 60 °C for 20 min.

Organosilanization with (3-Glycidyloxypropyl)trimethoxysilane. The procedure is analogous to the one reported above.

Poly(DMA) Graft Polymerization. A polymerization solution was prepared by dissolving *N*,*N*-dimethylacrylamide (1.0 M), cyanoisopropyl dithiobenzoate (5 mM), and AIBN (1 mM) in degassed and dry DMF. The slides derivatized with mercapto groups were dipped in this solution, under nitrogen atmosphere. The reactor containing the slides was sealed and the polymerization carried out at 60 °C in an oxygen-free environment. After 48 h, the slides were cooled and washed with DMF for 1 h at room temperature, with THF for 30 min, and with acetone for 30 min. They were then dried in an oven at 60 °C for 10 min.

Poly(DMA-b-GMA) Graft Polymerization. The grafted poly(DMA)−SC(S)Ph slides were placed in a reactor filled with a solution of 0.7 M GMA and 0.0065 M AIBN in dry and degassed DMF. The reactor was heated at 60 °C, and the polymerization was carried out for 16 h. Cooled slides were washed and dried as reported above.

Silica Bead Functionalization. Organosilanization with (3-Mercaptopropyl)trimethoxysilane. Silica beads, without any pretreatment, were incubated in a solution of 10% v/v (3-mercaptopropyl)trimethoxysilane in dry toluene for 12 h at room temperature under nitrogen and gentle stirring. Silanized silica particles were recovered, extensively washed with toluene, cyclohexane, and petroleum benzine, and then dried as described above. Polymer growth on silica was carried out as reported above.

Characterization of Functionalized Glass Slides. Tensiometry Measurements. Static contact angle measurements were carried out on microscope slides by an OCA20 instrument, by a sessile method according to the Young−Laplace equation.

Characterization of Functionalized Silica Beads. DRIFT Measurements. Diffuse reflectance measurements were made by dispersing coated silica beads in ground and dry KBr at a 10% w/w concentration. The samples were inserted in a 10-mm sample cup without applying any pressure. The surface was leveled off by a sharp-edged spatula. A total of 32 scans was measured in the range from 4000 to 400 cm^{-1} for each sample, at a resolution of 2 cm^{-1}. The spectra, in reflectance format, were converted in Kubelka−Munch units.

Functional Test of Microscope Glass Slides. Oligonucleotide Immobilization. A 23-mer, 5′-amine-modified oligonucleotide (100 mM stock solution, 5′-NH_2-$(CH_2)_6$-GCC CAC CTA TAA GGT AAA AGT GA) was dissolved in 150 mM sodium phosphate buffer at pH 8.5. A 10 μM solution of this oligonucleotide was printed on coated slides to form 10 × 10 spot subarrays using a QArray^2 spotter from Genetix (Hampshire, UK). Printed slides, in an uncovered storage box, were placed in a sealed chamber, saturated with NaCl, and incubated overnight at room temperature.

Hybridization Density. After spotting the slides with a 10 μM solution of 23-mer 5′-amino-modified oligonucleotide as reported above, the residual reactive groups of the coating were blocked by dipping the printed slides in 50 mM ethanolamine/0.1 M Tris pH 9.0 at 50 °C for 15 min. After discarding the blocking solution, the slides were rinsed two times with water and shaken for 15 min in 4× SSC/0.1% SDS buffer, prewarmed at 50 °C, and briefly rinsed with water.

An oligonucleotide, complementary to the one spotted on the surface, at a 1.0 μM concentration (2.5 $\mu L/cm^2$ of coverslip) was dissolved in the hybridization buffer (5× SSC, 0.1% SDS and 0.2% BSA) and immediately applied to microarrays. The complementary oligonucleotide has the sequence 5′-TCA CTT TTA CCT TAT AGG TGG GC-3′ and is labeled with Cy3 in 5′ position. The hybridized duplex has a melting temperature of 64.5 °C. This test allows us

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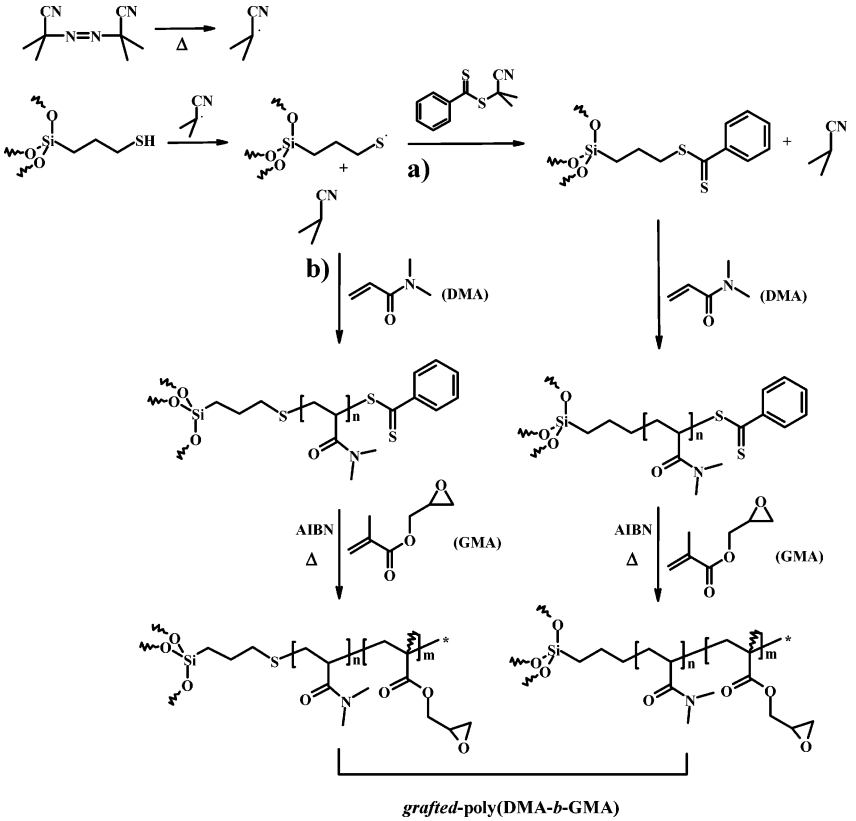
Scheme 2. Schematic Representation of the Hypothetical Mechanism Responsible for Block Brush Formation[a]



[a] Surface thiols, in the presence of a radical initiator (AIBN), form S• radicals that (pathway a) transesterify with cyanoisopropyl dithiobenzoate in solution or (pathway b) originate radical chains.

to evaluate the stability of the surface at high temperature, under conditions that are challenging for the coating. The slides, placed in a hybridization chamber, were transferred to a humidified incubator at a temperature of 65 °C for 4 h.

After hybridization, the slides were first washed with 4× SSC at room temperature to remove the coverslip and then with 2× SSC/0.1% SDS at hybridization temperature for 5 min. This operation was repeated two times and was followed by two washing steps with 0.2× SSC and 0.1× SSC for 1 min at room temperature. The slides were scanned with a Scan Array Express fluorescence scanner from Packard Bioscience at 22% laser power and 64% PMT.

The row data of spot fluorescence intensity were converted to molecules/cm^2 by using a standard curve of fluorescence made from a serial dilution of known amounts of fluorescent oligonucleotide (in the 0.025−15 μM concentration range). The data reported are replicates of 400 spots.

RESULTS AND DISCUSSION

An innovative procedure was devised to generate living chains on the surface of a glass slide. In this innovative process, the surface was, initially, derivatized with a thiol-bearing organosilane (γ-MPS) and then dipped in a solution of monomer (dimethylacrylamide) and initiator (AIBN) in the presence of a chain-transfer agent (cyanoisopropyl dithiobenzoate). Grafting of RAFT living chains commonly requires silanizing the surface with asymmetric organosilane RAFT agents or radical initiators. These types of molecules are difficult to synthesize, and the low yield of their reaction discourages their use in large-scale applications. In this work, we demonstrate that polymer brushes can be obtained by a RAFT process also with thiol molecules immobilized on the surface. A schematic representation of the hypothetical mechanism responsible for block brushes formation is reported in Scheme 2. Surface thiols, in the presence of a radical initiator (AIBN) form S• radicals that either transesterify with cyanoisopropyl dithiobenzoate in solution (pathway a) or originate radical chains (pathway b). Both pathways lead to formation of polymer chains initiated from the surface and terminated by a dithiobenzoate group according to the equilibrium reaction reported in Scheme 1. A significant portion of polyDMA chains are "living" and thus able to reinitiate polymerization to grow a second block of polyGMA. The growth of the second block has been confirmed by tensiometry, DRIFT spectroscopy, and functional tests.

Polymer Characteristics. Polydispersity and molecular weights of the polymers recovered from the solution are listed in Table 1. It's important to note that the polydispersity index (I_d) of polyDMA in solution is very close to unity, as reported for a living polymerization process. The polydispersity index, I_d, is the ratio of the weight-average molecular weight (M_w) to the number-average molecular weight (M_n). It indicates the distribution of individual molecular weights in a batch of polymers. According to Fukuda et al.,[47] we assume that molecular weights and polydispersity index of the free polymer are similar to those

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Table 1. Molecular Weights and Polydispersity[a] of Solution Polymers

polymer	$M_{n(expt)}$ "free" polymer (g/mol)	$M_{w(expt)}$ "free" polymer (g/mol)	I_d of "free" polymer
pDMA (brush)	3620	4270	1.18
pGMA (brush)	15620	40900	2.61

[a] Polymer molecular weights were determined by gel permeation chromatography (RI detector), by using HR 1, 2, 3 water and 4 columns in THF at 40 °C, relatively at polystyrene standards ranging from 1 to 1860 kDa ($I_d \sim 1$).

Table 2. Values of Static Contact Angles[a] for Different Surfaces Determined in Water

entry	group	solvent/ T (°C)	[CTA]/ [AIBN]	static CA (deg)/σ (N)[b]
1	glass			13.0/3.4 (17)
2	γ-GPS	THF/RT		56.7/1.8 (15)
3	thiol (MPS)	THF/RT		66.4/1.4 (17)
4	*g*-pDMA	DMF/60		56.0/1.8 (17)
5	*g*-pDMASC(S)Ph	DMF/60	5/1	72.0/2.8 (10)
6	*g*-p(DMA-*b*-GMA)	DMF/60		53.0/1.0 (12)

[a] SCA measurements were done by an OCA20 instrument, by a sessile method according to the Young−Laplace equation. [b] N indicates number of measurements and σ standard deviation on these.

of the grafted polymer. In the second step, the polyGMA growth proceeds without CTA molecules in solution; therefore, the only dithiobenzoate molecules present are those end grafted to polyDMA chains. Their concentration is too low to control the polydispersity, and this explains why a value higher than 2, which is typical for free radical polymerization, was obtained for polyGMA chains.

Surface Characterization by Physical Methods. *Tensiometry.* After each step of the derivatization process, a significant variation of the static contact angle value was observed, as reported in Table 2. The static contact angle in water of a surface coated with polyDMA grafted chains and not terminated by the CTA was 56° whereas that of a surface coated with polyDMA and terminated by a dithiobenzoate moiety was higher, up to 72° due to the hydrophobicity of this group. This is consistent with what reported in the literature[48] and is an indication of the presence of CTA at the end of grafted chains. The growth of a second block of polyGMA on polyDMA living chains was carried out by adding AIBN to a GMA solution in the absence of CTA. Also, in this case, the growth of the second block was monitored by contact angle variations. The contact angle found on slides in which the polyDMA block was reinitiated with a GMA monomer was 53°, a value slightly lower than that of the surface coated with a CTA-terminated polyDMA homopolymer. A reduction of the contact angle (from 72° to 53°) was expected due to the hydrophilic character of GMA and confirms the growth of a block of polyGMA.[49]

DRIFT Spectroscopy. Silica beads subjected to the coating procedure reported above were analyzed by DRIFT spectroscopy.

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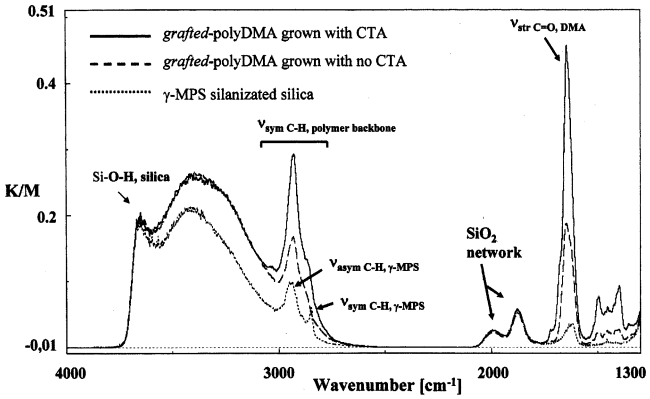

Figure 1. DRIFT spectra: overlapping of grafted polyDMA spectra, grown in the presence (—) and in absence (- - -) of CTA. The content of DMA is higher in the sample where the grafted polymer growth was mediated by the CTA molecules. The silica content, expressed by the intensity of the couple of peaks at 1877−1994 cm^{-1}, is constant in all spectra.

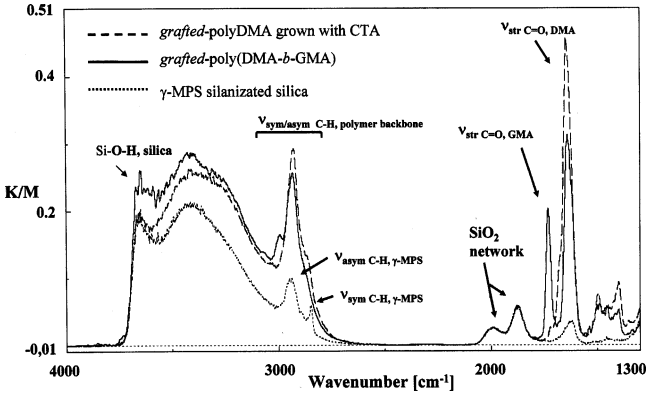

Figure 2. DRIFT spectra of polyDMA grown in the presence of CTA (- - -) and next grown with polyGMA (—).

The surface of silica was functionalized with a 10% v/v solution of mercaptosilane in THF. The growth of polyDMA in the presence and in the absence of cyanoisopropyl dithiobenzoate was monitored by DRIFT (Figure 1). In absence of diothiobenzoic ester, the mercapto groups immobilized on the surface produced a significant termination effect on the growth of solution chains (- - -line). Instead, when the polymerization was carried out in the presence of CTA (— line), the termination effect was significantly reduced, providing a more intense signal for the amide carbonyl stretching and for the C−H backbone stretching.

The growth of the second polyGMA block was also monitored by DRIFTS. In Figure 2, the spectra of the silica coated with polyDMA and with poly(DMA-*b*-GMA) (- - - line) are shown. The signal corresponding to the C=O stretching of methacrylate group (1731.7 cm^{-1}) is clearly visible in the spectrum of the poly(DMA-*b*-GMA)-coated silica. This is evidence of the growth of the second layer. To assess the molar ratio of the two monomers on the surface, two calibration curves were built for the two monomers, by depositing known amounts of polyGMA and polyDMA on known amounts of silica. As shown in Figure 3, the ratio between the peak area of C=O (ester and amide) and the characteristic signal of SiO_2 (couple of peaks) increased linearly with the amount of polymer deposited. The coated silica was stored in a LiBr humidity chamber for 24 h, to control the water content. The area of water signal, at 1630 cm^{-1} was added to that of carbonyl,

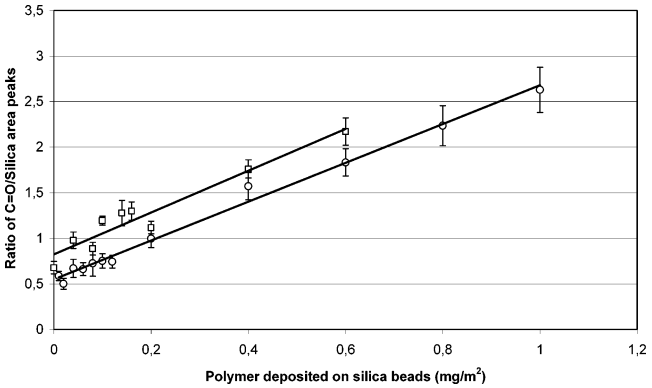

Figure 3. Calibration curves for (A) polyGMA and (B) polyDMA. Known amounts of the two polymers dissolved in chloroform were deposited on known amounts of silica. The ratio between C=O stretching area peak (at 1631.48 cm^{-1} for amide on polyDMA and 1731.7 cm^{-1} for ester on polyGMA) and SiO_2 network signal (area peak) at 1840−1994 cm^{-1} is plotted versus the amount of polymer deposited on silica beads.

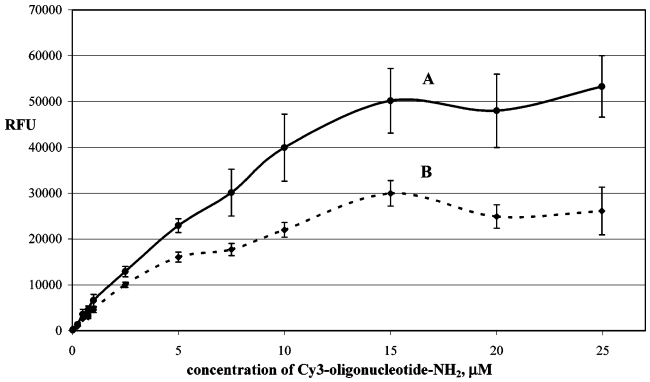

Figure 4. Cy3−oligonucleotide-NH_2 deposited at increasing concentration: (A) fluorescence measured after the spotting; (B) fluorescence remained on slide after washing (covalent linking).

keeping the humidity content constant. This explains why at zero concentration of polymer deposited, the ratio of the peak area is different from zero. Each measurement of the calibration curve was carried out on two replicates, and each sample was analyzed by DRIFTS at three different measurement angles (0°, 60°, 120°). The R^2 values for polyDMA and polyGMA calibration curves were 0.998 and 0.930, respectively.

By using these two calibration curves, the amount of polymer grafted to silica was calculated. Values of 0.923 mg/m^2 for polyDMA and 0.037 mg/m^2 for polyGMA, were determined. Assuming that the M_n values of the polymers in solution are representative of those grafted to the surface (47), a value of 0.24 pmol/cm^2 immobilized polyGMA was calculated. This value that corresponds to 1.4×10^{11} molecules/cm^2 and provides an indication on the number of polyDMA chains terminated by a dithiobenzoate group.

Surface Characterization by Functional Test. In a first test, increasing concentrations of a fluorescent oligonucleotide were arrayed on the surface and reacted with substrate. The covalent attachment of amino-modified oligonucleotide is due to the nucleophile addition of amine to the oxirane functionality[50] by the primary amino group of the oligonucleotide. This type of reaction is widely employed in the production of protein and DNA arrays and a number of commercial slides bearing oxirane groups are available. The fluorescence of the spots, 16 replicates for each concentration, after washing the unreacted excess of oligonucleotide was used to evaluate the binding sites saturation concentration. In Figure 4, the curves representing the fluorescence of the Cy3−oligonucleotide amino-modified recorded immediately after the spotting (curve A) and after washing the excess of unreacted molecules (curve B) are shown. For each concentration, above 1 μM, the fluorescence after washing is lower than that detected after spotting the oligonucleotide. In curve B, the fluorescence tends to a plateau when the oligonucleotide concentration is higher than 10 μM. Above this value, the binding sites are saturated; therefore, the amino-modified oligonucleotide probe was spotted at this concentration in the subsequent hybridization experiments.

The second functional test consists of a hybridization experiment and requires two steps: an amino-modified oligonucleotide was coupled overnight to the polymer brush, in a phosphate buffer at pH 8.5 at room temperature in a humid chamber (70% humidity). In the second step, a specific hybridization between the oligonucleotide arrayed on the surface and its complementary strand was carried out, by depositing on the glass slide a solution of the Cy3−oligonucleotide. After removing the excess of oligonucleotide solution, the slide was washed and scanned by a fluorescence detector. This test provides information on ODN density and accessibility. The amount of oligonucleotide bound to the surface upon hybridization was extrapolated from a fluorescence calibration curve built by depositing 0.2 nL of a Cy3 amino-modified oligonucleotide ranging in concentration from 0.025 to 15 μM.

Both functional tests were carried out either on a poly(DMA-*b*-GMA) coating or on a γ-GPS silanized surface. The different fluorescence obtained in identical experiments carried on the two surfaces clearly demonstrates that the density of accessible probes strictly depends on the characteristics of the coating. In a self-assembled monolayer, there is only one reactive group per silane molecule whereas in the brush block polymer coating, each segment of the GMA homopolymer contains several reactive groups (~100).

In Figure 5, a typical image of an ODN hybridization experiment carried out on the two surfaces is shown. The polymeric spacer between the long segment of polyGMA and the glass dramatically changes the binding capacity of the functional coating and the accessibility of the immobilized probes leading to a higher spot fluorescence intensity.

The number of oligonucleotide molecules linked to the surface and accessible for the hybridization, extrapolated from the calibration curve in the linear range between 0.025 and 5.0 μM (R^2 0.989), corresponds to 0.3×10^{13} molecules/cm^2. This value is based on the fluorescence of 400 spots whose average intensity was 15 400 arbitrary units of fluorescence. By combining the information provided by DRIFT on the number of polyGMA chains, 1.4×10^{11}/cm^2, with the oligonucleotide density given by the functional test, one can easily assume that, on average, each chain bears ~20 probes, which corresponds to about one oligonucleotide every five monomer residues.

(50) Tanaka, Y. In *Synthesis and Characteristics of Epoxides, Epoxy Resins: Chemistry and Technology*, May, C. A., Ed.; Marcel Dekker: New York, 1988.

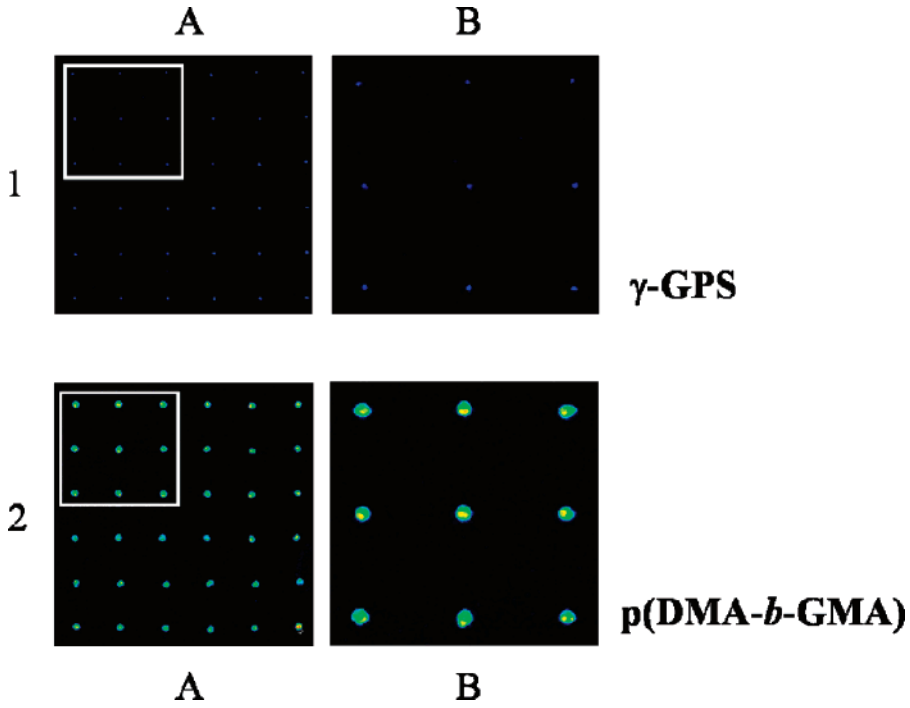

Figure 5. Oligonucleotide hybridization on GPS silanized slides (1) and poly(DMA-*b*-GMA) slides (2). The higher fluorescence obtained in (2) demonstrates that the density of accessible probes depends on the characteristics of the coating.

CONCLUSIONS

In this work, we provide an efficient sequential synthetic procedure to introduce block polymers on a glass surface by using RAFT process. A simple method, that involves silanization of glass by the commercial product γ-MPS, followed by the sequential introduction of two different polymeric layer was proposed. The functional surface obtained was characterized by tensiometry and DRIFTS. Both techniques demonstrated the presence of the block copolymer of the desired composition whereas a functional test based on the hybridization of complementary strands of DNA allowed us to quantify the density of immobilized and hybridized molecules. A comparison between a surface silanized with 10% w/v γ-GPS and a block polymer obtained by silanization shows that the performance provided by the polymeric coating is superior than that of the organosilanized surface.

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