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Synthesis and Characterization of Surface-Grafted Polyacrylamide Brushes and Their Inhibition of Microbial Adhesion

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A method is presented to prevent microbial adhesion to solid surfaces exploiting the unique properties of polymer brushes. Polyacrylamide (PAAm) brushes were grown from silicon wafers by atom transfer radical polymerization (ATRP) using a three-step reaction procedure consisting of immobilization of a coupling agent γ-aminopropyltriethoxysilane, anchoring of an ATRP initiator 4-(chloromethyl)benzoyl chloride, and controlled radical polymerization of acrylamide. The surfaces were characterized by X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy, ellipsometry, and contact-angle measurements. The calculated grafting density pointed to the presence of a dense and homogeneous polymer brush. Initial deposition rates, adhesion after 4 h, and detachment of two bacterial strains (*Staphylococcus aureus* ATCC 12600 and *Streptococcus salivarius* GB 24/9) and one yeast strain (*Candida albicans* GB 1/2) to both PAAm-coated and untreated silicon surfaces were investigated in a parallel plate flow chamber. A high reduction (70–92%) in microbial adhesion to the surface-grafted PAAm brush was observed, as compared with untreated silicon surfaces. Application of the proposed grafting method to silicone rubbers may offer great potential to prevent biomaterials-centered infection of implants.

Introduction

Microbial adhesion is a serious complication after the insertion of biomaterials implants or devices in the human body[1] and depends on the physicochemical surface properties of the adhering microorganisms and the biomaterial.[2] Consequently, a variety of different surface modifications has been developed, based on both physical and chemical techniques, to discourage microbial adhesion. The wettability of biomaterial surfaces, for instance, can be increased by plasma treatment, but this effect is most often only transient[3] and effective inhibition of microbial adhesion is absent after several months. Also, fluorination[4,5] in order to create more hydrophobic surfaces has been demonstrated to affect microbial adhesion. Although encouraging under laboratory conditions, usually showing reductions in microbial adhesion by a factor 2 or 3, these reductions have overall been too small to be significant under clinical conditions except under conditions of high shear, as in the human oro-pharyngeal cavity.

Polyethylene oxide (PEO) has been promoted often in biomedical applications to prevent protein adsorption to surfaces. For example, PEO has been bonded to polystyrene and low-density polyethylene surfaces by use of electron beam irradiation,[6]

incorporated into a polyurethane matrix material,[7] or chemically grafted to a surface.[8] Also, the adsorption of proteins and adhesion of mammalian cells were highly reduced by covalent attachment of zwitterionic polymers such as poly(sulfobetaine methacrylate) and poly(carboxybetaine methacrylate).[9]

Polymer chains attached by one end to a surface or interface with a density of attachment points high enough to obligate the chains to stretch along the normal to the surface or interface are so-called polymer brushes, as opposed to the so-called mushroom regime. Polymer brushes increase the distance between microorganisms and a substratum surface by entropic effects, therewith reducing the attractive forces between surface and the microorganisms.[10–12] As a result, reductions in microbial adhesion by 2 orders of magnitude have been reported[13] for different strains and species.

Polymer brushes can be prepared on surfaces either by grafting to, in which case reactive polymer end groups react with active sites on the surface, or by grafting from, in which case a surface-immobilized initiator is used to grow polymer chains directly from the surface by polymerization.[14,15] High grafting densities and relatively thick layers are difficult to realize with the grafting to technique due to the repulsive forces between polymer chains that are already attached and non-grafted polymer chains that are entering. Radical polymerization, and especially atom transfer radical polymerization (ATRP), is a very suitable procedure to grow polymer brushes from a surface because it allows a good

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(1) Gristina, A. G. *Science* **1987**, *237*, 1588–1595.
(2) Hermansson, M. *Colloids Surf. B* **1999**, *14*, 105–119.
(3) Everaert, E. P. J. M.; Van der Mei, H. C.; De Vries, J.; Busscher, H. J. *J. Adhes. Sci. Technol.* **1995**, *9*, 1263–1278.
(4) Everaert, E. P. J. M.; Van der Mei, H. C.; Busscher, H. J. *Colloids Surf. B* **1998**, *10*, 179–190.
(5) Silver, J. H.; Lin, J. C; Lim, F.; Tegoulia, V. A.; Chaudhury, M. K.; Cooper, S. L. *Biomaterials* **1999**, *20*, 1533–1543.
(6) Sofia, S. J.; Merrill, E. W. *J. Biomed. Mater. Res.* **1998**, *40*, 153–163.
(7) Lee, J. H.; Ju, Y. M.; Lee, W. K.; Park, K. D.; Kim, Y. H. *J. Biomed. Mater. Res.* **1998**, *40*, 314–321.
(8) Archambault, J. G.; Brash, J. L. *Colloids Surf., B* **2004**, *33*, 111–120.
(9) Zhang, Z.; Chao, T.; Chen, S.; Jiang, S. *Langmuir* **2006**, *22*, 10072–10077.
(10) Halperin, A. *Langmuir* **1999**, *15*, 2525–2533.
(11) Morra, M. *J. Biomater. Sci.: Polym. Ed.* **2000**, *11*, 547–569.
(12) Bos, R.; Van der Mei, H. C.; Busscher, H. J. *FEMS Microbiol. Rev.* **1999**, *23*, 179–230.
(13) Roosjen, A.; Kaper, H. J.; Van der Mei, H. C.; Norde, W.; Busscher, H. J. *Microbiol.* **2003**, *149*, 3239–3246.
(14) Luzinov, I.; Julthongpiput, D.; Malz, H.; Pionteck, J.; Tsukruk, V. V. *Macromolcules* **2000**, *33*, 1043–1048.
(15) Farhan, T.; Huck, W. T. S. *Eur. Polym. J.* **2004**, *40*, 1599–1604.

control over the grafting density and brush thickness.[16] In order to obtain a polymer brush with a good resistance against microbial adhesion, three parameters should be taken into consideration: (i) the grafting density, (ii) the brush thickness, and (iii) the hydrophilicity of the grafted film. The transition from the brush to mushroom regime has been suggested in the case of grafted polyacrylamide chains to occur at a grafting density of ~0.065 nm^{-2} for grafted chains of a $\bar{M}_n = 17\,000$ g mol^{-1}.[17] At low grafting densities, when the polymer chains are in the mushroom regime, the thickness of the polymer layer is independent of the grafting density. However, in a brush regime, at high polymer chain densities, the thickness increases with increasing the molecular weight of the polymer. Jones and co-workers[18] reported that the initiator density on a surface was associated with the final polymer grafting density. Using ω-mercaptoundecyl bromoisobutyrate to initiate the radical polymerization of methyl methacrylate from a gold-covered surface, a linear relationship between the initiator density and polymer grafting density was found.

It is the aim of this paper to investigate a new method to graft dense polyacrylamide (PAAm) brushes on silicon wafers, as a model surface for silicone rubber. The efficiency of the brush in reducing microbial adhesion is evaluated for two bacterial strains and a yeast strain. PAAm was used because it is a biocompatible, water soluble (it has no upper or lower limits), polar, electrically neutral and stable polymer. Although it is known that photochemically immobilized PAAm on silicone rubber and ethylene—propylene rubber surfaces suppresses the adsorption of fibrinogen and immunoglobulin G and also inhibits fibroblast growth,[19,20] such effects have never been described for microbial adhesion.

Materials and Methods

Materials and Reagents. Silicon wafers (125 mm diameter, 900 μm thickness, both sides polished, 1−1-1 orientation and phosphorus doped to 1000 Ω cm resistivity) were supplied by Topsil Semiconductors Materials A/S (Frederikssund, Denmark) and cut into 2.5 cm × 2 cm samples. γ-Aminopropyltriethoxysilane (APS), 4-(chloromethyl)benzoyl chloride (CMBC), acrylamide (AAm), 2,2′-dipyridyl (byp), copper(I) chloride, and benzyl chloride (BC) were purchased from Aldrich. All solvents were reagent grade and used without further purification.

Surface Preparation and Modification. In order to remove dust particles and organic contaminations, the silicon surfaces were ultrasonically rinsed with methanol, acetone, and dichloromethane for 10 min each and subsequently dried under vacuum. The surfaces were additionally cleaned and hydrophilized by UV/ozone treatment using an UV/ozone photoreactor PR-100 (Uvikon) during 30 min and placed about 5 mm from the UV lamp. The reactor is equipped with a low-pressure mercury-quartz lamp to generate UV radiation (185 and 254 nm). During the UV exposure period, the 185 nm line dissociates the atmospheric oxygen and generates ozone while the 254 nm line dissociates the ozone and initiates the cleaning process.[21]

The chemical bonds from organic contaminations were broken under a high energy of UV light and degradative oxidation to H_2O and CO_2 took place (eq 1):

$$\text{Hydrocarbon} + (O_2, O_3, h\nu) \rightarrow H_2O + CO_2 \qquad (1)$$
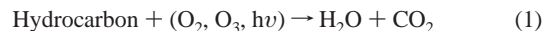
This treatment reduced the water contact angle of the surface from $45° \pm 1°$ to $10° \pm 2°$.

Polyacrylamide brushes were introduced on the surface, immediately after the cleaning process according to reaction Scheme 1.

Aminosilanization was carried out in a 2% (v/v) solution of APS in toluene for 1.5 h at room temperature. APS molecules in an organic surrounding can condense with each other and form patches consisting of polymerized APS.[22,23] APS belongs to the short-alkyl-chains category which tends to form multilayers, while the long-alkyl-chains have the tendency to form a monolayer.[24] The silanized silicone surfaces were washed with toluene in a Soxhlet for 24 h[22] and finally baked under vacuum at 120 °C for 30 min. The samples were stored under dry nitrogen atmosphere.

To immobilize the ATRP initiator, CMBC, the aminosilanized surfaces were immersed in a 2% (w/v) solution of CMBC in dichloromethane for 1 h at 50 °C. Afterward, the functionalized silicon wafers were washed with dichloromethane for 15 min to remove any unreacted material. Finally, the surfaces were dried under vacuum and stored under dry nitrogen atmosphere.

For surface-grafting of PAAm brushes by ATRP, a flat-bottom flask containing the initiator-functionalized silicon surfaces was deoxygenated by several vacuum-nitrogen cycles. The polymerization of AAm was performed in N,N-dimethylformamide (DMF), as reported by Wu et al.[25] The solution containing 1.78 g (25 mmol) of AAm, 0.025 g (0.25 mmol) of Cu(I)Cl, and 0.08 g (0.5 mmol) of byp in 10 mL of DMF was stirred for 5 min under dry nitrogen atmosphere to form a dark-brown solution. The reaction mixture was then transferred with a syringe to the flask containing the functionalized surfaces and the temperature was raised to 130 °C. After 48 h the silicon wafers were removed from the solution and washed with deionized water for 48 h in a Soxhlet apparatus to remove any unreacted monomer, catalyst and non-grafted material. Finally, the surfaces were dried under vacuum at 110 °C for 30 min.

Synthesis of Non-Grafted PAAm via ATRP. In order to estimate the molecular weight of the surface-grafted PAAm brushes by means of gel permeation chromatography (GPC), AAm was polymerized in solution using BC as the ATRP "free initiator". The reaction was carried out under similar experimental conditions as the surface-grafting polymerization. The molar ratio was kept 1:100:1:2 (BC/AAm/Cu(I)Cl/byp). Thus, 15 g (200 mmol) of AAm, 0.2 g (2 mmol) of Cu(I)Cl, and 0.62 g (4 mmol) of byp were introduced in a 250 mL round-bottom flask equipped with a magnetic stirrer. First, a nitrogen stream was passed over the reaction mixture for 10 min. Next, the DMF was added and the reaction mixture was stirred under nitrogen for another 10 min. Finally, 0.24 mL (2.08 mmol) of BC was added via a degassed syringe under continuous stirring and the temperature was raised to 130 °C for 48 h. The dark-brown reaction solution turned green on exposure to air which indicated the oxidation of Cu (I) to Cu (II). The Cu (II) catalyst was removed by passing the reaction solution through a silica column and PAAm from the resulting colorless solution was precipitated in a 20-fold excess of acetone. The white precipitate was filtered and the residue was dried in a vacuum oven at 50 °C overnight.

Physico-Chemical Surface Characterization. The silicon wafer surface was characterized prior to and after modification by means of X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), ellipsometry, contact-angle, and atomic force microscopy (AFM) measurements. The molecular weight of PAAm was determined by gel permeation chromatography (GPC).

(16) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921−2990.

(17) Wu, T.; Efimenko, K.; Genzer, J. *J. Am. Chem. Soc.* **2002**, *124*, 9394−9395.

(18) Jones, D. M.; Brown, A. A.; Huck, W. T. S. *Langmuir* **2002**, *18*, 1265−1269.

(19) DeFife, M. K.; Shive, S. M.; Hagen, K. M.; Clapper, D. L.; Anderson, J. M. *J. Biomed. Mater. Res.* **1999**, *44*, 298−307.

(20) Mirzadeh, H.; Katbab, A. A.; Khorasani, M. T.; Burdof, R. P.; Gorgin, E.; Golestani, A. *Biomaterials* **1995**, *16*, 641−648.

(21) Zafonte, L.; Chiu, R. *Proc. SPIE−Int. Soc. Opt. Eng.* **1984**, *470*, 164−175.
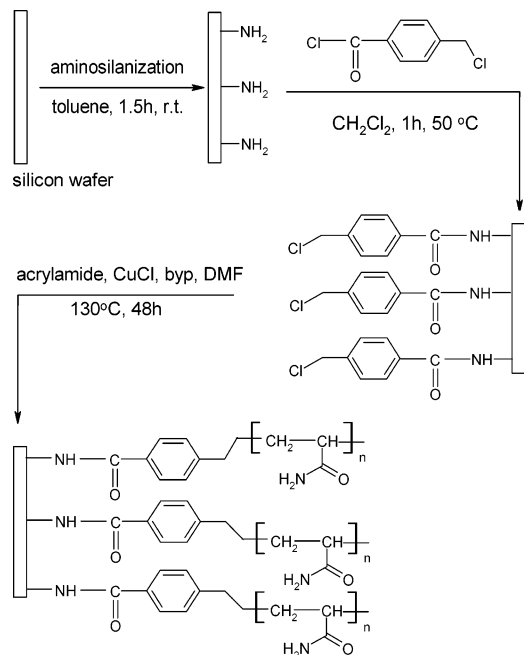
(22) Simon, A.; Cohen-Bouhacina, T.; Porté, M. C.; Aimé, J. P.; Baquey, C. *J. Colloid Interface Sci.* **2002**, *251*, 278−283.

(23) Wieringa, R. H.; Siesling, E. A.; Geurts, P. F. M.; Werkman, P. J.; Vorenkamp, E. J.; Erb, V.; Stamm, M.; Schouten, A. J. *Langmuir* **2001**, *17*, 6477−6484.

(24) Xiao, S. J.; Textor, M.; Spencer, N. D. *Langmuir* **1998**, *14*, 5507−5516.

(25) Wu, T.; Tomlinson, M.; Efienko, K.; Genzer, J. *J. Mater. Sci.* **2003**, *38*, 4471−4477.

Scheme 1. Schematic Representation of Silicon Wafer Surface Modification by Means of APS Synthesis, ATRP Initiator Attachment, and PAAm Grafting

Table 1. Elemental Surface Compositions of the Silicon Wafer after Each Modification Step as Determined by XPS and Theoretical Composition of PAAm

element	UVO (%)	APS (%)	CMBC (%)	PAAm (%)	PAAm, experimental	PAAm, theoretical
O (1s)	49.6	31.8	24.6	23.5	24.9	20
C (1s)	–	38.6	41.7	53.3	56.5	60
N (1s)	–	6.2	6.0	17.6	18.6	20
Cl (2p)	–	–	5.9	–	–	–
Si (2s)	50.3	23.4	21.8	5.6	–	–

XPS spectra were recorded on a Surface Science Instrument SSX-100 photoelectron spectrometer with a monochromatic AlK$_\alpha$ X-ray source (hν = 1486.6 eV). Measurements were carried out at a photoelectron take off angle of 35° to the sample surface. The resolution of the wide scans was set to 4 and the acquisition of C$_{1s}$ signal was done at constant pass energy of 50 eV.

The film thickness was measured with a Nanofilm EP3 imaging ellipsometer. The wavelength of the laser beam was 532 nm and the angle of incidence was set to 70°. A model based on three organic layers with characteristic values for the refractive indices and thicknesses was used to calculate the thickness of the brush.

Transmission FTIR measurements were performed on a Bruker IFS 66 v/S spectrometer equipped with a DTGS detector. All spectra are averages of 100 scans measured at a resolution of 4 cm^{-1}. The spectrum of a clean silicon wafer was used as a reference.

Advancing water contact angles were measured at room temperature with a Krüss DSA-10 MK2 drop shape analysis system. The drop size was 0.5 μL, which was kept constant for all measurements. All angles reported are averages over three separately prepared samples, while on each sample three droplets, placed at different positions, were measured.

The surface morphology of the substrate-grafted films was studied with a Digital Instruments Nanoscope IIIa Multi Mode system. The device was equipped with a scanner with a maximum scan range of 10 μm in x and y and 2.5 μm in z. AFM images were obtained in the tapping mode.

The molecular weight of PAAm synthesized was determined with a Viscoteck GPC Max solvent delivery system equipped with a degasser, autosampler, autoinjector and a TDA 302 triple detection system. First the PAAm samples were dissolved in water and filtered over a 0.45 μm filter. 0.2 mol NaNO$_3$ per liter solution was used as the mobile phase with a flow rate of 0.5 mL min^{-1}. Aliquots of 100 μL of the polymer solution were injected through two 300 × 6 mm TSK GMPW XL columns at 30 °C.

Culture Conditions and Microbial Adhesion. Two bacterial strains (*Staphylococcus aureus* ATCC 12600 and *Streptococcus salivarius* GB 24/9) and one yeast strain (*Candida albicans* GB 1/2) were used to evaluate the effects of the brushes in preventing microbial adhesion. All strains were first cultured for 24 h at 37 °C in strain specific growth media. Tryptone soya broth (OXOID, Basingstoke, England) was used for *S. aureus*, Todd Hewitt broth (OXOID) for *S. salivarius* and brain heart infusion broth (OXOID) for *C. albicans*.

Each culture was used to inoculate a second culture for 16 h. The microorganisms were harvested by centrifugation for 5 min at 5,000 g for *S. aureus* and 5 min at 10 000*g* for *S. salivarius* and *C. albicans* and washed twice with PBS (10 mM potassium phosphate, 140 mM NaCl, pH 6.8). *S. aureus* and *S. salivarius* were sonicated on ice for 10 s and suspended in 200 mL PBS at a concentration of 3×10^8 mL^{-1} for bacteria and 3×10^6 mL^{-1} for yeast.

A parallel plate flow chamber was used to study initial microbial adhesion and detachment. Inside, the flow chamber is equipped with a stainless steel bottom plate (7.6 × 4.9 cm), which has a special hole (2 × 1 × 0.1 cm) to place the samples in. At regular intervals, adhering organisms were observed with a CCD−MXR camera (High Technology, Eindhoven, The Netherlands), mounted on a metallurgical microscope (Olympus BH-2) equipped with a 40× ultralong working distance objective (Olympus ULWD-CD Plan 40 PL) used for experiments with bacteria and a 10× objective (Olympus ULWD-CD Plan A10 PL) for yeast. The images were recorded with an image analyzer (TEA, Difa, Breda) and evaluated on the computer.

Prior to each experiment, all air bubbles were removed by filling the tubes with PBS and passing it through the flow chamber. Subsequently, a microbial suspension was recirculated through the system for 4 h with hydrostatic pressure at the shear rate of 10 s^{-1}. The yeast suspension was continuously stirred to avoid microbial sedimentation. The initial increase in the number of adhering microorganisms with time, was expressed in a so-called initial deposition rate j_0 (cm^{-2} s^{-1}), i.e., the number of adhering microorganisms per unit area and time. The number of microorganisms adhering after 4 h, n_{4h}, was taken as an estimation of microbial adhesion in a more advanced state of the process. At the end of each experiment, a single air-bubble was passed through the system, which is known to exert a high detachment force on adhering micron-sized particles and its effect on the number of adhering bacteria was determined. All values presented are the averages of experiments on three separately prepared brush-coated surfaces and were carried out with separately grown microorganisms.

Results and Discussion

Physico-Chemical Surface Characterization of the PAAm Brushes. The XPS spectrum of the silicon surface after UV/ozone (UVO) treatment shows only Si and O, each with a contribution to the surface composition of ∼50% (Table 1). No C signal could be detected confirming the cleanliness of the surfaces. After aminosilanization (APS), new peaks appear at a binding energy of 400 and 285 eV, attributed to nitrogen and carbon, respectively. The C$_{1s}$ peak (Figure 1a) is composed of a C−C component at 284.8 eV (71.4%) due to aliphatic hydrocarbons, a C−N component at 286.0 eV (14.9%), and a C-O component at 286.4 eV (13.7%) from the ethoxy end groups. For an APS monolayer, the C/N ratio should be 3 if all ethoxy groups after hydrolysis had reacted with the surface. The atomic percentages from XPS show an excess in C as compared to the N, which can be a result of unreacted ethoxy groups on the surface. This is also indicated by the presence of the C−O peak in the C$_{1s}$ core level region. The unreacted ethoxy groups might be due to the dry reaction conditions employed during the APS immobilization on the silicon wafer surface.
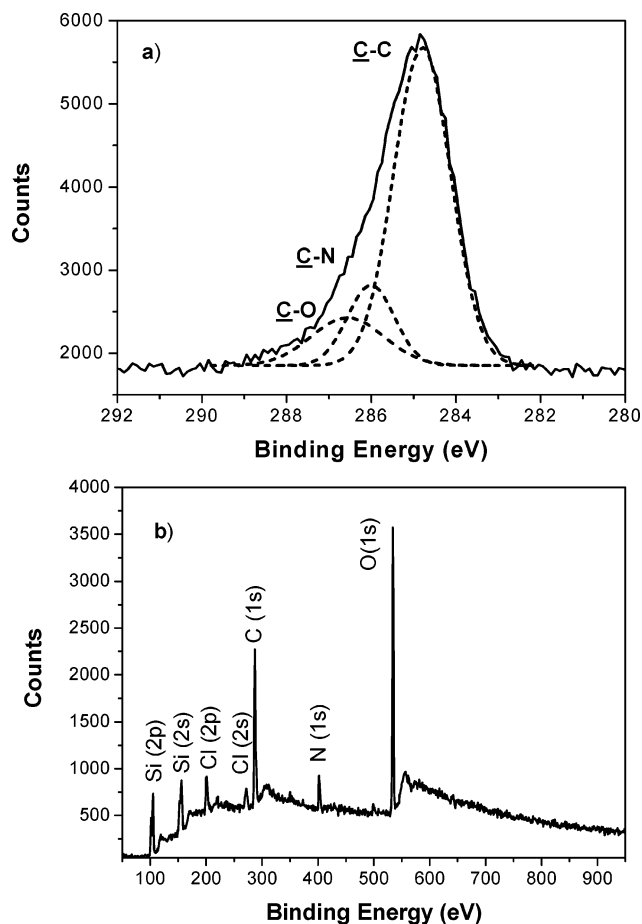
Figure 1. XPS spectra of the silicon surface (a) after aminosilanization-C_{1s} core level region and (b) after immobilization of the ATRP initiator-CMBC.

Figure 1b shows the XPS spectrum of the CMBC initiator layer. From the Cl/N ratio, the degree of substitution can be estimated: assuming one CMBC group per amino group (i.e., complete conversion), the Cl/N ratio should be 1. In practice, the ratio varies from 0.55 to 0.95 calculated from 20 samples.

The presence of a PAAm layer on the surface can be inferred from a strong decrease in the measured Si (2s) content from 50.3% to 5.6%, (see Figure 2a and Table 1) and an increase in the amount of surface nitrogen to 17.6%. The presence of a Si peak, in spite of high polymer thickness as determined by ellipsometry, can be attributed to the formation of the polymer domains during the heating process as shown in Figure 4b. The C_{1s} core level region also indicates the formation of a polymeric layer (see Figure 2b) from its components at 288 eV (21%) attributed to amide carbon CO—N and 284.8 eV (71.7%) C—C assigned to aliphatic hydrocarbon. The peak at 286.4 eV (7.3%) is considered to be a contribution of the APS layer based on the presence of the Si peaks in the wide spectra (Figure 2a). The contributions of C (1s), O (1s), and N (1s) to the surface composition of experimental PAAm in Table 1 were calculated with the XPS software by not taking into consideration the contribution of Si peaks. The theoretical composition of PAAm and experimental values from XPS summarized in Table 1 matches quite well. The higher experimental oxygen and carbon contents are believed to belong to layers beneath PAAm. Also the higher oxygen content can result from atmospheric contamination.[26] The absence of the copper in the XPS spectra indicates that the ligand-catalyst system was effectively removed during the washing procedure after the polymerization reaction.
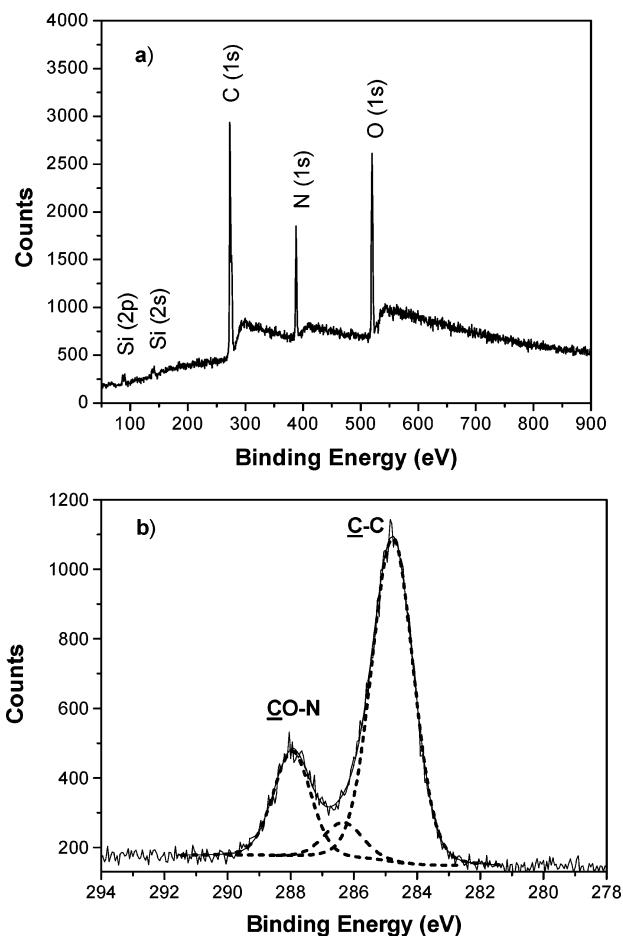

Figure 2. XPS spectra of the silicon surface after atom transfer radical polymerization of AAm (a) wide scan and (b) C_{1s} core level region.
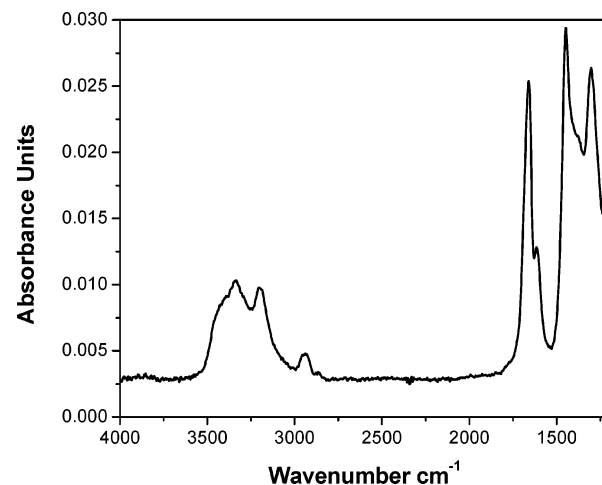

Figure 3. Transmission FTIR spectrum of a surface-grafted PAAm brush on a silicon wafer.

Ellipsometry indicated that the native SiO_2 layer had a thickness of 1.5 nm and a refractive index of 1.46. The thickness of each additional organic layer was calculated by subtracting the contribution of the previous layer. Refractive indices for APS and PAAm of 1.42 (taken from the Sigma-Aldrich catalog) and 1.54,[27] respectively, were used. The refractive index of CMBC was calculated with the software of the ellipsometer to be 1.59.

(26) Cecchet, F.; Leigh, D. A.; Rapino, S.; Margotti, M.; Paolucci, F.; Baggerman, J.; Brouwer, A. M.; Kay, E. R.; Wong, J. K. Y.; Rudolf, P. *J. Phys. Chem. B* **2004**, *108*, 15192−15199.
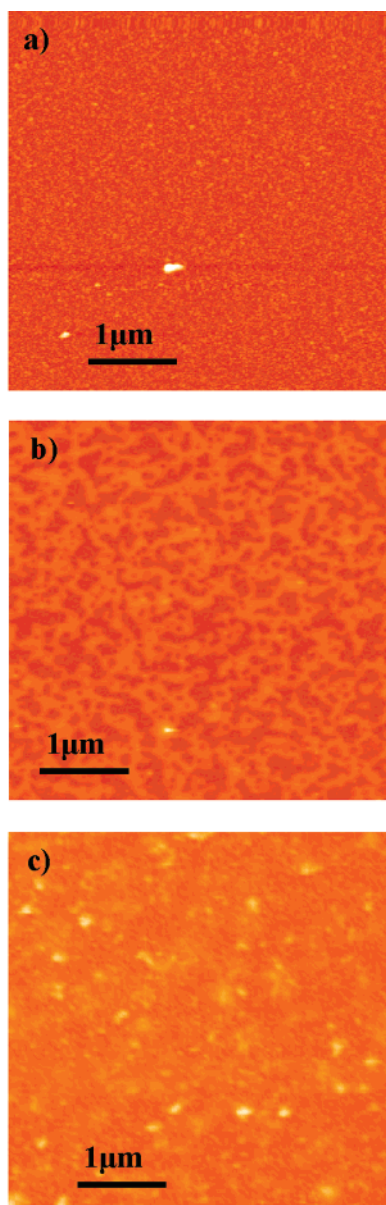
Figure 4. Tapping mode images ($4 \, \mu m \times 4 \, \mu m$) of (a) grafted APS on silicon wafer after the UVO cleaning procedure; (b) grafted PAAm dried at high temperature; and (c) grafted PAAm dried at room temperature.

On the basis of this model, we determined an APS layer thickness of 0.9 ± 0.2 nm indicating monolayer formation. The CMBC layer thickness was 1.6 ± 0.9 nm and the PAAm layer was approximately 20 ± 2 nm thick.

The FTIR absorption spectrum of the silicon wafers after PAAm grafting reveals several bands characteristic for PAAm. The absorption bands from Figure 3 at 3334 cm^{-1} and 3198 cm^{-1} are attributed to the stretching modes of the amine functionality and the signals at 1660 cm^{-1} and 1617 cm^{-1} are assigned to the amide I and amide II vibration mode, respectively. The peak at 2934 cm^{-1} is mainly due to the νC$-$H of the polymer chain.

Upon aminosilanization, the water contact angle of the silicon wafers increased from $10° \pm 2°$ to $59° \pm 3°$. This effect is due to the nature of APS: it contains a hydrophobic sequence (propyl chain) with an amino end group. The surface hydrophobicity remained similar ($58° \pm 3°$) after the introduction of the CMBC

initiator layer but decreased dramatically after the grafting of PAAm to $28° \pm 5°$.

The surface morphology of the surface-grafted PAAm film can be studied with AFM. A very flat and smooth surface with a root-mean-square roughness (rms) value of 0.7 nm for a $2 \, \mu m \times 2 \, \mu m$ scan area resulted after the cleaning process. Figure 4a shows an AFM image of an APS layer on a silicon substrate after Soxhlet extraction with toluene giving a surface roughness of 1 nm. The number of large bright spots corresponding to small aggregates resulting from siloxane oligomerization is very low. Figure 4b shows an AFM image of a PAAm-coated silicon wafer. The sample was dried after the washing process under vacuum at a temperature of 110 °C for 30 min. Figure 4c is the image resulted after the PAAm brush coating from silicon wafer and dried under vacuum at room temperature. The Z range of the scans in both cases b and c is 20 nm and an increase in rms value after the PAAm grafting up to 3.4 nm is a result of the polymeric layer formation.

The destabilization of the polymer film in domains spread over the surface after the sample drying at high-temperature (Figure 4b) can be caused by the forces resulted from the confinement of the thermal molecular motion in the film.[28] Heating the polymer above its glass transition temperature (Tg) can cause collapsing of the polymer and the surface is dewetted leading to the formation of the polymer droplets. It has been shown that Tg of PAAm after annealing at 180 °C for 2 h occurs at 199 °C[29] for a chain nanoglobules obtained by spray-drying of a solution of PAAm with $M_w = 5 \, 10^6$ g mol^{-1}. Other studies reported T_g values of solid PAAm close to 188 °C.[30] The molecular weight of PAAm in our experiments is ($Mw = 41700$ g mol^{-1}) and the temperature used to dry the polymer film is 110 °C much lower than the Tg of PAAm to cause the dewetting of the polymer. However, the presence of water might result in a decrease in Tg and cause the polymer dewetting.

PAAm Characteristics. The number-average molecular weight of the final polymer from solution using the "free initiator" was determined by GPC as $\bar{M}_n = 26\,500$ g mol^{-1}. During the grafting polymerization from silicon wafer surfaces, a white precipitate appeared in the dark-brown solution which indicated the formation of free PAAm. Knowing the molecular weight of the polymer, the thickness of the polymer layer in the dry state ($h = 20 \pm 2$ nm) and the bulk density of PAAm ($\rho = 1.302$ g cm^{-3}) the grafting density (σ) of the polymer chains can be calculated using eq 2, yielding to 0.6 nm^{-2}, in the dry state:

$$\sigma = h\rho N_A / \bar{M}_n \qquad (2)$$

The grafting density can be also defined as a function of the distance between grafted points (D):[14,31]

$$\sigma \equiv a^2 / D^2 \qquad (3)$$

The size of an isolated chain in a good solvent is given by the Flory radius

$$R_{Fl} \sim a N^{3/5} \qquad (4)$$

where N is the number of monomer units and a is the statistical segment length.

(27) Huang, X.; Doneski, L. J.; Wirh, M. J. *Anal. Chem.* **1998**, *70*, 4023−4029.

(28) Steiner, U. *J. Polym. Sci.* **2005**, *43*, 3395−3405.
(29) Mi, Y.; Xue, G.; Lu, X. *Macromolecules* **2003**, *36*, 7560−7566.
(30) Mark, H. F., Bikales, N. M., Overberger, C. G., Menges, G., Eds. *Encyclopedia of Polymer Science and Engineering*, 2nd ed.; Wiley: Indianapolis, IN, 1985; Vol. 1, pp 169−211.
(31) Auroy, P.; Auvray, L.; Léger, L. *Phys. Rev. Lett.* **1991**, *66*, 719−722.
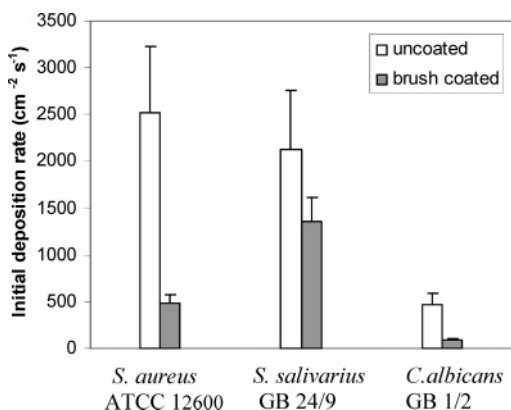
Figure 5. The microbial initial deposition rates (j_0) on uncoated and PAAm brush coated-silicon wafers. All error bars represent the average standard deviation obtained from three separate experiments.

The critical grafting density (σ^*) appears at the limit between the mushroom and brush regime where the distance between the two grafted chains is approximately R_{Fl} ($D = R_{Fl}$)

$$\sigma^* \equiv a^2/D^2 \equiv R_{Fl}^2/(N^{6/5}D^2) \equiv N^{-6/5} \tag{5}$$

The critical grafting density for PAAm with $\bar{M}_n = 17\,000$ g mol^{-1} ($N = 239$) is $\sigma^* \equiv 0.0014$. The separation between the mushroom and brush regimes in water as solvent for this molecular weight was determined experimentally to occur at $\sigma_{calc}^* = 0.065$ nm^{-2}.[17] Further, we consider that $\sigma_{calc}^* = C \times \sigma^*$ and we use the coefficient $C = 46.43$ to determine the crossover for a grafted PAAm chain in water with $\bar{M}_n = 26\,500$ g mol^{-1} ($N = 373$). Thus, the critical grafting density is $\sigma^* \equiv 373^{-6/5} \sim 0.0008$ and the calculated transition between brush and mushroom regime in water takes place at $\sigma_{calc}^* = C \times 0.0008 = 0.037$ nm^{-2}.

The calculated grafting density in dry state (0.6 nm-2) of PAAm chains having $\bar{M}_n = 26\,500$ g mol^{-1} is well above the threshold for the brush regime in water (0.037 nm^{-2}) for the same $\bar{M}_n$.

It has been shown that the maximum coverage of a silicon wafer with OH groups[32] is 5 OH groups nm^{-2} and the maximum surface coverage with NH$_2$ groups[33] from the silanization step is 2.5 NH$_2$ groups nm^{-2}. We assume that in our experiments the surface coverage with OH and NH$_2$ groups is maximal. The degree of conversion of NH$_2$ groups into Cl groups was estimated to be between 55 and 95% from the Cl/N ratio measured with XPS, which means that the density of the Cl groups varies between 1.4 and 2.4 nm^{-2}. From the calculated grafting density of PAAm on the surface (0.6 nm^{-2}) we can conclude that one out of four initiators bound to the surface started a polymer chain. The situation where 1 out of 10 initiators had been used to start a polymer chain has been reported before.[18,34] The effect was ascribed to the mutual steric hindrance of the growing polymer chains because the diameter of a polymer chain backbone $\sim$1.8–2.0 nm^2 is much larger than the surface area of an initiator $\sim$0.2–0.5 nm^2. In our case a precipitation polymerization occurs because PAAm is insoluble in DMF, and the polymer chains are in the collapsed state. This might lead to blocking of some initiator sites.

Microbial Adhesion Experiments. Figure 5 shows the initial deposition rate (j_0) of all microorganisms used for adhesion experiments. The different microbial strains adhere with different

(32) Zhuravlev, L. T. *Langmuir* **1987**, *3*, 316−318.
(33) Moon, J. H.; Shin, J. W.; Kim, S. Y.; Park, J. W. *Langmuir* **1996**, *12*, 4621−4624.
(34) Kim, J. B.; Bruening, M. L.; Baker, G. L. *J. Am. Chem. Soc.* **2000**, *122*, 7616−7617.
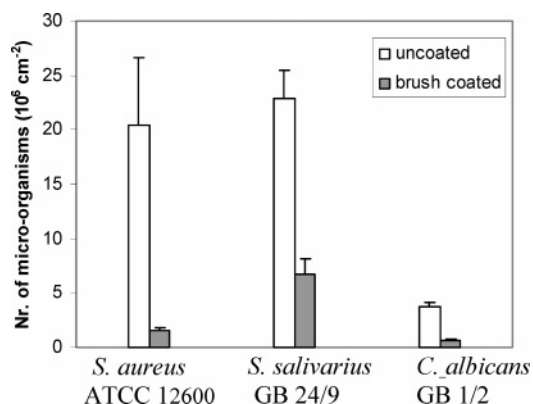

Figure 6. Adhesion of microorganisms to uncoated and PAAm-coated silicon wafer surfaces after 4 h in a parallel plate flow chamber at room temperature. All error bars represent the average standard deviation obtained from three separate experiments.

Table 2. Reduction in Microbial Adhesion to PAAm-Coated Silicon Surfaces Relative to Uncoated Surfaces after 4 h of Flow at Room Temperature, and the Detachment Created by Passing an Air Bubble through the Flow Chamber for PAAm-Coated and Uncoated Surfaces

microbial strains	adhesion reduction (%)	detachment from PAAm-coated surface (%)	detachment from uncoated surface (%)
S. aureus ATCC 12600	92 ± 4	65 ± 2	17 ± 4
S. salivarius GB 24/9	70 ± 9	87 ± 7	11 ± 0.3
C. albicans GB 1/2	83 ± 7	100 ± 0.2	99 ± 1

initial rates. The initial deposition of *S. aureus* and *S. salivarius* on uncoated silicon wafer surface is similar while the deposition of *C. albicans* is slower due to its larger size. The initial deposition rates decrease for all brush coated surfaces. *S. salivarius* deposited fastest on brushes with an initial deposition rate of 1352 cm^{-2} s^{-1} whereas the deposition of the other strains vary from 488 cm^{-2} s^{-1} for *S. aureus* to 90 cm^{-2} s^{-1} for *C. albicans*.

After 4 h (Figure 6), all microorganisms deposit less on brush-coated silicon wafers as compared with their deposition on non-coated samples. The highest value was obtained for *S. salivarius* (6.7 × 10^6 cm^{-2}) and the lowest number for *C. albicans* (0.6 × 10^6 cm^{-2}). The percentage reduction in microbial adhesion after 4 h on the brush is summarized in Table 2, demonstrating reduction between 1 and 2 log-units for the various strains. The strength of the adhesion forces between the microorganisms and the surface can be estimated from the percentages of microorganisms detached by passing an air−water interface (so-called air-bubble) over the surface (Table 2). Air-bubble passage removed 65% of the *S. aureus* strain from the brush and only 17% from uncoated silicon. *S. salivarius* was the strain with a higher adhesion to the brush after 4 h of flow, but after air-bubble passage a considerably high number was removed from the brush as compared with its removal from bare silicon. The yeasts were detached almost completely from both brush and non-coated surfaces, from which we concluded that the adhesion strength of the yeasts was far less than of the bacterial strains involved in this study.

Conclusions

PAAm brushes were successfully grafted from silicon wafer surfaces using an APS coupling layer. The presence of PAAm on the surface was demonstrated by FTIR and XPS results. This grafting method has the advantage of obtaining a dense brush regime as determined by the thickness of the polymer film and

the grafting density. 1 out of 4 initiator molecules starts the growing of a polymer chain being an improvement of the results obtained before where only 1 out of 10 initiator molecules result in a grafted polymer chain. The absence of the copper in the XPS spectra makes this procedure to be suitable for biomedical applications. Here for the first time it was shown that grafting a densely packed and covalently attached PAAm brush layer from the surface of silicon wafer reduces the attractive forces between the surface and microorganisms. A strong reduction in microbial adhesion was seen on PAAm brush-coated surfaces for all strains, whereas organisms that did adhere to the brush did so through weak adhesion forces.