Research article

SURFACE and INTERFACE ANALYSIS

Received: 21 July 2010 Revised: 29 December 2010 Accepted: 3 January 2011 Published online in Wiley Online Library: 26 January 2011

(wileyonlinelibrary.com) DOI 10.1002/sia.3741

Controlled adhesion of *Salmonella Typhimurium* to poly(oligoethylene glycol methacrylate) grafts

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Poly(oligoethylene glycol methacrylate), POEGMA, brushes were prepared by surface-initiated atom transfer radical polymerization (SI-ATRP) on gold-coated silicon wafers. Prior to ATRP, the substrates were grafted by brominated aryl initiators via the electrochemical reduction of a noncommercial parent diazonium salt of the formula BF_4^-, $^+N_2$-C_6H_4-$CH(CH_3)Br$. The diazonium-modified gold plates (Au-Br) served as macroinitiators for ATRP of OEGMA which resulted in hydrophilic surfaces (Au-POEGMA) that could be used for two distinct objectives: (i) resistance to fouling by *Salmonella Typhimurium*; (ii) specific recognition of the same bacteria provided that the POEGMA grafts are activated by anti-Salmonella. The Au-POEGMA plates were characterized by XPS, polarization modulation-infrared reflection-absorption spectroscopy (PM-IRRAS) and contact angle measurements. Both Beer-Lambert equation and Tougaard's QUASES software indicated a POEGMA thickness that exceeds the critical ~10 nm value necessary for obtaining a hydrophilic polymer with effective resistance to cell adhesion. The Au-POEGMA slides were further activated by trichlorotriazine (TCT) in order to covalently bind anti-Salmonella antibodies (AS). The antibody-modified Au-POEGMA specimens were found to specifically attach *Salmonella Typhimurium* bacteria. This work is another example of the diazonium salt/ATRP process to provide biomedical polymer surfaces.

Keywords: gold; diazonium salts; ATRP; POEGMA; bacteria; bioadhesion; XPS; PMIRRAS; contact angles; AFM

Introduction

It is well known that organic polymer coatings permit to tune the surface properties of materials such as wetting,[1] adhesion,[2] chemical sensing;[3,4] response to chemical, temperature, pH, ionic strength, light, and mechanical stimuli;[5] cell adhesion,[6] resistance to protein adsorption[7] and bacterial fouling,[8] to name but a few.

Particularly, there is a growing demand for antifouling polymers in order to prevent the formation of biofilms by pathogenic bacteria.[8,9] In this regard, polyacrylates, oligosaccharides, poly(sulfobetaine methacrylate)s, poly(ethylene glycol)-based polymers, and polyglycidol are well known to be effective in resisting nonspecific protein or cellular adhesion.[10] Nevertheless, hydrophilic polymers such as poly(hydroxyethyl methacrylate) [PHEMA],[8] poly(hydroxypropyl methacrylate) [PHPMA][11] or polyglycidol[12] can be activated by proteins and antibodies in order to switch them from nonfouling to bioadhesive coatings. Indeed, the pendant OH groups from antibacterial PHEMA grafts prepared on titanium surface were converted into carboxyl or amine groups to allow for the coupling of gentamicin, penicillin, or collagen via the carbodiimide chemistry.[9] The antibiotic-modified grafts retained antibacterial properties whereas the collagen-modified ones favoured fibroblast and osteoblast cell adhesion and proliferation. Tugulu *et al*.[13] modified antifouling PHEMA and POEGMA brushes by RGD-containing peptide ligands for the specific adhesion of human umbilical vascular endothelial cells.

In this work, we report on the preparation of polymer brushes that have controlled interfacial interactions with *Salmonella Typhimurium*, a well known pathogenic bacterium which can survive in the environment under starvation-stressful conditions for up

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to ten months.[14] The polymer grafts were prepared by atom transfer radical polymerization (ATRP) initiated by diazonium-modified substrates instead of the traditional methods employing grafted initiators from self assembled monolayers of thiols on gold[7] or silanes on glass.[8] Towards this end, ATRP initiators were electrografted to gold via the electrochemical reduction of the noncommercial diazonium salt BF_4^-, $^+N_2$-C_6H_4-$CH(CH_3)Br$ to provide Au-C_6H_4-$CH(CH_3)Br$ plates (Au-Br) serving as macroinitiators for the ATRP of oligoethylene glycol methacrylate. The gold-grafted poly(oligoethylene glycol methacrylate) (Au-POEGMA) plates and reference materials were characterized by XPS, polarization modulation-infrared reflection-absorption spectroscopy (PM-IRRAS) and contact angle measurements. The polymer-modified substrates were further activated in order to attach anti-Salmonella (AS) via the trichlorotriazine coupling procedure. Adhesion of *Salmonella Typhimurium* to Au-POEGMA plates and their antibody-modified form, Au-POEGMA-AS, was evaluated by AFM.

Experimental

Materials

Gold coated silicon wafers with a thickness of about 1000 Å were used as substrates (purchased from Aldrich). The wafers were cut into slides of 1 × 1.5 cm. Just before use, to remove the organic residues on the surface, the slides were ultrasonically rinsed with acetone, water and ethanol, dried in a stream of argon, then cleaned in an UV surface decontamination system (Novascan, PSD-UV) and rinsed with acetonitrile.

Oligo(ethylene glycol) methacrylate (OEGMA, 360 g/mol, average number of ethylene oxide repeat units ~6, Aldrich) was purified by removing inhibitor by filtering through silica column, and stored under an argon atmosphere at −4 °C before use.

2,2-bipyridine (bpy) was from Alfa Aesar; copper (I) bromide (CuBr), copper (II) bromide ($CuBr_2$), 1,3,5-trichlorotriazine (TCT) and 1-aminoethanol were Aldrich products. *N*,*N*-dimethylformamide (DMF), dichloromethane (DCM), acetonitrile (ACN), methanol (MeOH), phosphate-buffered saline (PBS, 10 mM, pH 7.4) were all of analytical reagent grade from Acros Organics and were used as received. Deionized water was purified by a Millipore water purification system.

Salmonella enterica serovar *Typhimurium* LT2 DT104 obtained from LATVPEP Lab (Faculty of Pharmacy, Monastir) was cultured in LB agar for 24 h and the cells were grown at 37 °C in LB broth for 24 h. *S. Typhimurium* LT2 DT104 cells were washed twice by centrifugation (3500 rpm for 10 min at 20 °C) with 10 ml PBS, then repeating centrifugation and suspension in 2 ml PBS. The final pellet was suspended in 5 ml of PBS (10^6 colony-forming units/ml).

Rabbit polyclonal anti-Salmonella (Ab13634) was purchased from Abcam Inc. (Cambridge, MA) as a solution of 4.5 $mg.ml^{-1}$ concentration.

Synthesis of the diazonium salt BF_4^-, $^+N_2$-C_6H_4-$CH(CH_3)Br$

The synthesis of the starting diazonium salt D1 was conducted according to Matrab *et al*.[15] ^1H NMR (200 MHz, $CDCl_3$), δ ppm : 3.67 (q, 1H), benzylic protons; 2.07 (d, 3H), methylic proton; 7.90 (d, 2H) and 8.50 (d, 2H), aromatic protons β to the diazonium function and to the benzylic carbon, respectively.

Electrochemical reduction of the diazonium salt D1 on gold surface

Electrografting aryl groups was performed with an Autolab PGSTAT 30 potentiostat using a purpose-built three-electrode cell comprising a gold slide (the substrate to be modified) as working electrode, a platinum counter electrode, and a SCE (saturated calomel electrode) reference electrode operated at room temperature. The electroreduction of the diazonium salt D1 was carried out in a degassed solution of ACN containing 5 mM of D1 and 0.1 M of supporting electrolyte NBu_4BF_4 for 5 min. Cyclic voltammetry measurements was used to determine the reduction peak position of D1. Samples for surface-initiated ATRP were prepared by chronoamperometry by setting the potential at −700 mV (*vs* SCE). After modification, the slides were rinsed by sonication in acetonitrile, ethanol then dichloromethane, and subsequently dried under a stream of argon. The so modified substrates are, in the following, abbreviated by Au-Br.

Surface-initiated ATRP of oligoethylene glycol methacrylate

CuBr (180 mg, 1.26 mmol), $CuBr_2$ (14 mg, 0.063 mmol), bpy (490 mg, 3.15 mmol) powders were added into the external tube of the dried Schlenk reactor (100 ml) equipped with a magnetic stir bar and topped with a refrigeration unit using cool water circulation. The Au-Br slides were introduced into the internal tube, sealed with a rubber septum then deoxygenated with argon. OEGMA (17.5 g, 48.6 mmol) was dissolved in a mixture of methanol (3.5 ml) and H_2O (14 ml) and the resulting solution bubbled with argon for 20 min. This solution was injected via a purged cannula into the Schlenk reactor. The polymerization was carried out at 60 °C under argon for 3 h; it was stopped by exposing the catalyst to air. During ATRP, there was no loss of solvent by evaporation since it continuously recondensed once the vapors reached the refrigeration unit. The modified gold slides were taken out, thoroughly washed with DMF and H_2O, followed by Soxhlet-extraction in ethanol overnight to remove the unreacted monomer and any other organic species. Au-POEGMA slides were dried and stored under argon.

Grafting POEGMA via SI-ATRP on gold is successful, as will be demonstrated below by XPS. We have conclusively shown for poly(methyl methacrylate), PMMA, that a physisorbed PMMA is easily removed by simple ultrasonically assisted solvent wash.[16] Growth of polymer chains on substrates through aryl layers is thus an efficient and reliable means of preparation of metal–polymer joint, a situation that holds in the actual Au-POEGMA case.

Covalent immobilization of *Salmonella Typhimurium* antibody onto Au-POEGMA

Covalent immobilization of *Salmonella Typhimurium* antibody onto POEGMA brushes was performed according to Basinska *et al*.[12] The hydroxy groups of POEGMA chains were activated with TCT, by incubating Au-POEGMA in a solution of 1.1 g of TCT dissolved in a dioxane/water (15 ml/15 ml) mixture which was stirred for 4 h at room temperature. Afterwards, the activated surfaces were thoroughly washed with dioxane.

The TCT-activated POEGMA chains were incubated in a solution of polyclonal anti-Salmonella prepared by adding 100 µl of the as-received antibody sample to 40 ml of PBS.

The samples were incubated overnight and slides with immobilized bacteria antibodies were purified from unbound proteins in solution by washing and storing in fresh PBS. About

0.25 ml of solution of 1-aminoethanol (Aldrich) was added to the solution containing the antibody-treated slides in order to saturate binding sites not occupied by bacteria antibodies. After incubation for 3 h, the excess of 1-aminoethanol was removed by three repeated washings with PBS and the slides were stored in the buffer.

Bacterial strain and growth conditions

Salmonella enterica serovar *Typhimurium* LT2 DT104 obtained from LATVPEP Lab (Faculty of Pharmacy, Monastir) was cultured in LB agar during 24 h at 37 °C. Then, the cells were washed three times by centrifugation (3500 rpm for 10 min at 20 °C) with 10 mlL PBS (pH 7). The final pellet was suspended in 5 ml of PBS.

Colony-forming unit (CFU) determination

The number of viable cells in each dilution was determined by spread plating 0.1 ml of each dilution on duplicate slides of trypticase soja agar (TSA) which were incubated at 37 °C for 48 h. The number of colonies (CFU/ml) was determined between 30 and 300 colonies/plate. The tubes with diluted cells were immediately placed on ice and used for testing with untreated and modified Au substrates.

XPS

The spectra were recorded using a Thermo VG Scientific ESCALAB 250 system fitted with a microfocused, monochromatic Al Kα X-ray beam (1486.6 eV, 650 µm spot size). The samples were stuck on sample holders using conductive double-sided adhesive tapes and pumped overnight in the fast entry lock at $\sim 5 \times 10^{-8}$ mbar prior to transfer to the analysis chamber (typically less than 5×10^{-9} mbar). The *Avantage* software, version 3.51, was used for digital acquisition and data processing. The pass energy was set to 150 and 40 eV for the survey and narrow regions, respectively. Additional high-resolution C1s regions were recorded using 15 eV pass energy. The spectra were calibrated against the $Au4f_{7/2}$ peak set 84 eV. However, in the case of Au-POEGMA, whilst Au substrate exhibited a negligibly small charging effect, the top polymer layer had a positive static charge of 0.4 eV which was subtracted from the polymer core level peak positions. The surface composition was determined using the integrated peak areas and the corresponding Scofield sensitivity factors corrected for the analyzer transmission function. Additional peak fittings were performed using peak components of comparable full width at half maxima and Lorentzian shape in the 0–30% range.

PM-IRRAS

PM-IRRAS spectra were recorded with a Nicolet 860 FTIR (Thermo-Electron) spectrometer with a resolution of 8 cm^{-1} by adding 2000 scans with an optical mirror velocity of 0.474 cm^{-1}/s. The spectrometer was equipped with a commercially available module (Thermo Electron) including a ZnSe photoelastic modulator (PEM) and measurements were performed at an incident angle of 80° under a (partially) dry atmosphere. After reflection on the sample, the incident beam is focused with a ZnSe lens on a MCT-A detector cooled at 77K.

Contact angle measurements

Water contact angles were examined with a Krüss DSA100 instrument (Hamburg, Germany), a drop shape analyzer. The plates were placed in a thermostat-controlled chamber at 25.0 ± 0.1 °C. The drops were left to interact with the slides for 15 min, a period over which the contact angles were automatically measured every 5 s. For each sample, we performed measurements of three to five droplets put on different spots of the substrate surface.

AFM

Uncoated and bacteria-coated gold slides were imaged by a Nanoscope III Digital Instrument in the tapping mode using a Si_3N_4 tip cantilever. The cantilever oscillation frequency was set at 320 kHz. Tips of the cantilever were characterized by the radius of their curvature, which was equal to 7 ± 2 nm. No computer filtering procedure was used to treat the images. Tapping mode imaging were recorded with 256 pixels per line with a scan rate of 1.2 Hz. Surface roughness (Ra) was determined at the same scale (1 µm) for each sample.

Results and discussion

General strategy for the preparation of Au-POEGMA hybrids

The protocol for preparing antifouling Au-POEGMA hybrids and their activation in view of immobilizing bacteria is schematically shown in Fig. 1. Hereafter, the specimens are abbreviated by Au for clean gold-coated silicon wafers; Au-Br for gold grafted with $-C_6H_4-CH(CH_3)Br$ initiator; Au-POEGMA for Au-Br after surface-initiated ATRP of OEGMA; Au-POEGMA-TCT for Au-POEGMA treated by TCT; and Au-POEGMA-AS for Au-POEGMA-TCT activated by anti-*Salmonella*.

We shall first consider the electrochemical attachment of the aryl layer to gold and then tackle the surface modification of the supports by ATRP and further surface chemistry for the attachment of polyclonal anti-Salmonella. We will then compare the interactions of *Salmonella Typhimurium* with Au, Au-POEGMA and Au-POEGMA-AS.

Electrografting of the ATRP initiators to gold

The noncommercial D1 is readily electrochemically reduced on gold surfaces to yield attached $-C_6H_4-CH(CH_3)-Br$ (1) groups for surface-initiated ATRP. Cyclic voltammetry was used for the determination of the electrochemical reduction potential, found at Epc = −0.5 V/SCE which corresponds to the reduction of the diazonium salt (see similar approach with another diazonium salt in Ref. [4]). Chronoamperometry (Fig. 2) was used to prepare Au-Br by maintaining the Au electrodes for 300 s at a potential of −700 mV, reference SCE. The very steep decrease of the current with time is characteristic of the formation of the organic layer which hampers the electron transfer from the electrode. The electrochemically treated gold slides (Au-Br) were then thoroughly rinsed under sonication in deaerated acetonitrile.

Surface chemical composition

XPS

The surface chemical compositions of the uncoated and modified gold substrates were assessed by XPS. Figure 3 shows the survey

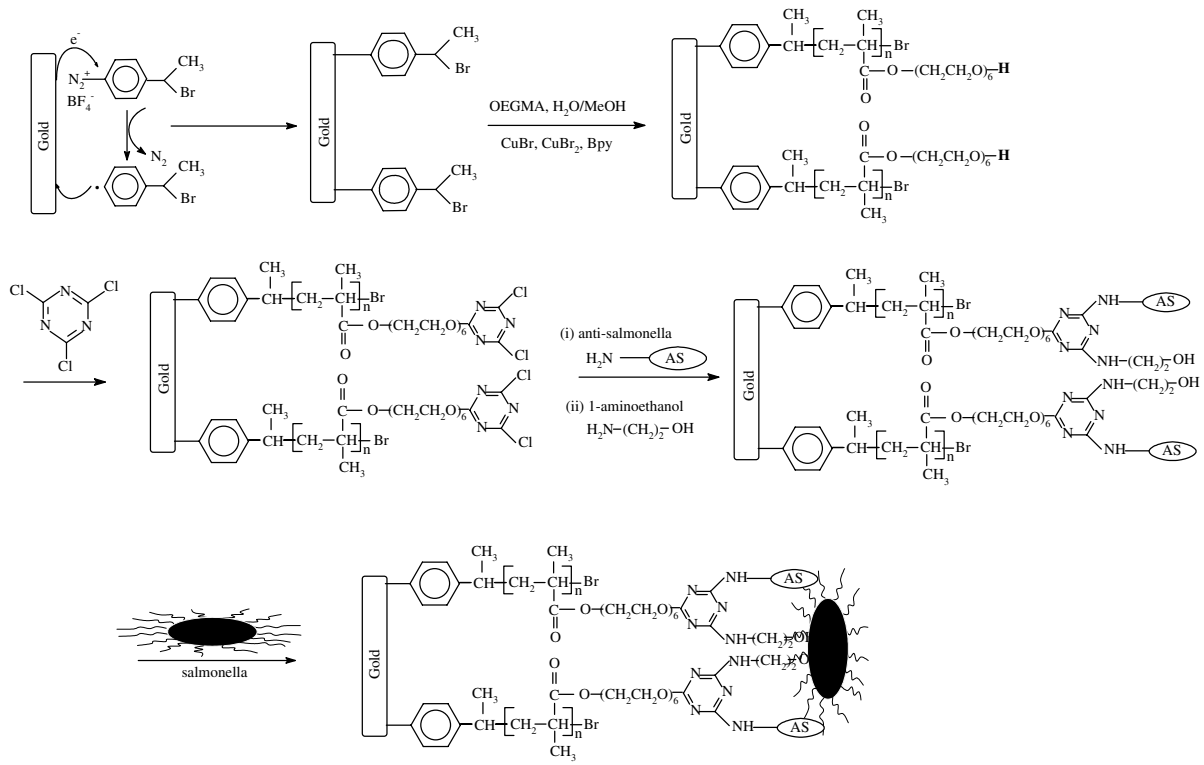

Figure 1. Preparation of Au-POEGMA hybrids and their activation by covalently attached polyclonal anti-*Salmonella* for the specific recognition of *Salmonella Typhimurium*.

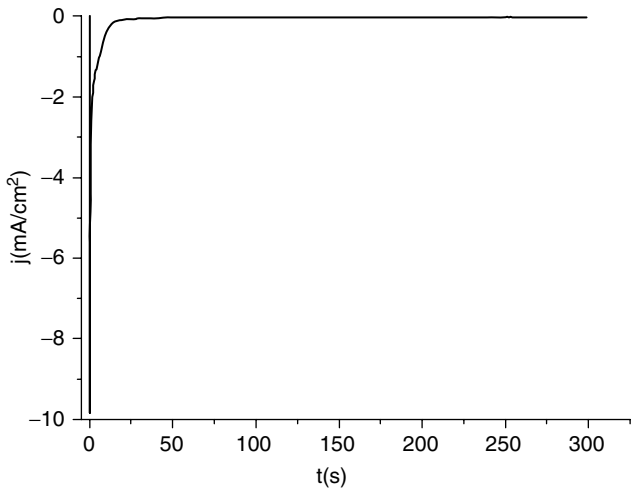

Figure 2. Chronoamperometry of a gold slide grafted with 5 mM **D1** in ACN + 0.1 M NBu_4BF_4, 700 mV negative potential, $t = 300$ s, $v = 0.2$ V s^{-1}. Reference SCE.

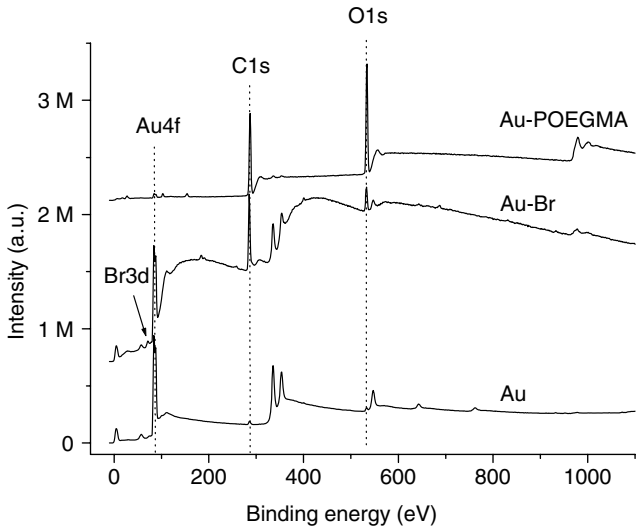

Figure 3. XPS survey spectra of Au, Au-Br and Au-POEGMA.

scans of Au, Au-Br and Au-POEGMA. The main peaks C1s, O1s and $Au4f_{7/2}$ are centered at 285, 533 and 84 eV, respectively. The electrografting of the initiator moieties results in a low-intensity Br3d peak visible at ~71 eV. Whilst Au and Au-Br surfaces are dominated by Au4f, the Au-POEGMA hybrid exhibits sharp O1s and C1s peak. The attenuation of Au core-hole peaks is reflected in the increase of the background of the inelastically scattered electrons in the Au-Br spectrum. In the case of Au-POEGMA, the spectrum shows an Au4f doublet of low intensity, a qualitative indication of a successful ATRP process for the preparation of thin POEGMA films. Note the absence of Br3d peak which suggests a high average degree of polymerization of OEGMA.

Table 1 reports the surface elemental composition (in atomic ratios) as determined by XPS. Upon grafting the aryl layer and then the POEGMA thin film, the Au/C ratio gradually decreases. For O/C, the ratio decreases from 0.27 (Au) to 0.11 (Au-Br) as the first grafted aryl layer contains essentially C, H and Br. For the Au-POEGMA plates, the O/C is 0.45 close to the theoretical value 0.5 expected for POEGMA which contains 6 ethylene oxide groups

Table 1. Apparent surface composition of Au, Au-Br and Au-POEGMA as determined by XPS

Materials	Au/C	O/C	Br/C
Au	1.94	0.27	–
Au-Br	0.10	0.11	0.02
Au-POEGMA	3.3×10^{-3}	0.45	0

in each OEGMA repeat unit. Nevertheless, an O/C ratio close to the expected one for pure POEGMA, and the massive attenuation of Au core-level peaks, bring strong supporting evidence for a dense overlayer of tethered POEGMA chains.

The high-resolution C1s regions from Au-Br and Au-POEGMA are displayed in Fig. 4. The substrate Au-Br has a C1s peak fitted with 6 components centered at 284.8 (C-C/C-H), 285.8 (C-Br), 286.5 (C-O), 287.8 (C═O), 289.1 (O-C═O) and 291.2 eV ($\pi \rightarrow \pi^*$ shakeup satellite transition). The latter is a fingerprint of the attached aryl groups.[1] The C1s shakeup satellite and the Br3d peak centered at ~71 eV testify for the successful electrografting of the -C_6H_4-CH(CH_3)Br ATRP initiator groups.

In the case of Au-POEGMA, the high-resolution C1s peak exhibits a fine structure similar to that reported elsewhere for POEGMA prepared by ATRP on silicon wafers.[17] The peak is fitted with three main components centered at 285.0, 286.5 and 289.0 eV corresponding to C-C/C-H, C-O and O-C═O chemical environments. The main component (C-O bonds) is assigned to the oligo(ethylene glycol) segments in the OEGMA repeat units.

The POEGMA thickness can further be determined either by classical approaches that combine the peak attenuation of gold substrate by the top layers. Alternatively, one can analyze the change in background structure in a wide energy region[18] using the QUASES software [www.quases.com] to estimate the thickness. In the following, and for the sake of simplicity, it is assumed that the aryl intermediate layer and POEGMA graft make a single organic top layer.

Using the classical Beer-Lambert equation $I = I^{\circ} \exp(-d/\lambda \cos\theta)$, the aryl layer is found to have a thickness of 6.6 nm comparable to that obtained by Bernard *et al.*[19] for -C_6H_4-I on gold. After ATRP of OEGMA the whole organic layer is 20.6 nm thick which means that POEGMA has a thickness of 14 nm. Assuming a density of 1 g/cm^3 for organic polymers, POEGMA has a grafting density of 14 mg/m^2.

Using the QUASES software, we determined the thickness of the aryl and the POEGMA grafts. The lower panel in Fig. 5 shows the analysis of the Au4f peak from the Au-Br sample. The best fit is obtained for Au covered by a 3.4-nm-thick aryl layer, a thickness that differs from that determined by Beer-Lambert equation but which, nevertheless, remains within the range generally reported in the literature.[19] Sun *et al*. estimated by ellipsometry infrared spectroscopy the thickness of 4-carboxyphenyl layer on gold to be in the 2.5–5 nm range.[20]

QUASES was also used to determine the thickness of the aryl/POEGMA bilayer following the approach described by Gam-Derouich.[1] The background in the O1s region of Au-POEGMA was fitted assuming a mean free path of 2.7 nm[21] and using the Polymer cross-section.[22] The best fit, shown in the upper panel of Fig. 5, was obtained for oxygen distributed over a depth between 0.5 and 26 nm. The 0.5-nm-thick top layer would be due to an oxygen-free adventitious contamination. By difference with the thickness of the aryl layer, one would end up with a ~22-nm-thick POEGMA layer. It is important to stress that both methods (QUASES and the traditional Beer-Lambert) lead to a top layer thicker than the critical value of 9.5 nm[17] which accounts for resisting protein adsorption.

PM-IRRAS

Figure 6 shows the PMIRRAS spectra of Au-Br and Au-POEGMA. Au-Br spectrum exhibits two bands at 1512 and 1604 cm^{-1} characteristic of the aromatic rings, confirming the presence of grafted aryl moieties at the gold surfaces. In addition, the absence of N≡N diazonium salt stretching band at 2257 cm^{-1}, confirms the cleavage of N_2 molecule during the electroreduction process and the covalent grafting of the aryl layers. The weak band at 1110 cm^{-1} could be attributed to the C-N elongation vibration.

For Au-POEGMA, the spectrum shows the appearance of two strong bands at 1730 and 3425 cm^{-1}, attributed to the stretching vibration of C═O ester groups, and OH groups, respectively. The increased intensity of the peak centered at 1120 cm^{-1}, is assigned to the presence of stretching vibrations of POEGMA C-O-C groups. One also can notice the presence of strong symmetric and antisymmetric CH_2 stretching peaks at 2967 and 2876 cm^{-1}, respectively, which confirms the grafting of the organic chains.

Hydrophilic/hydrophobic characteristics of gold slides

The hydrophilic/hydrophobic properties of the Au, Au-Br and Au-POEGMA slides were examined by water contact angles (θ_w). The

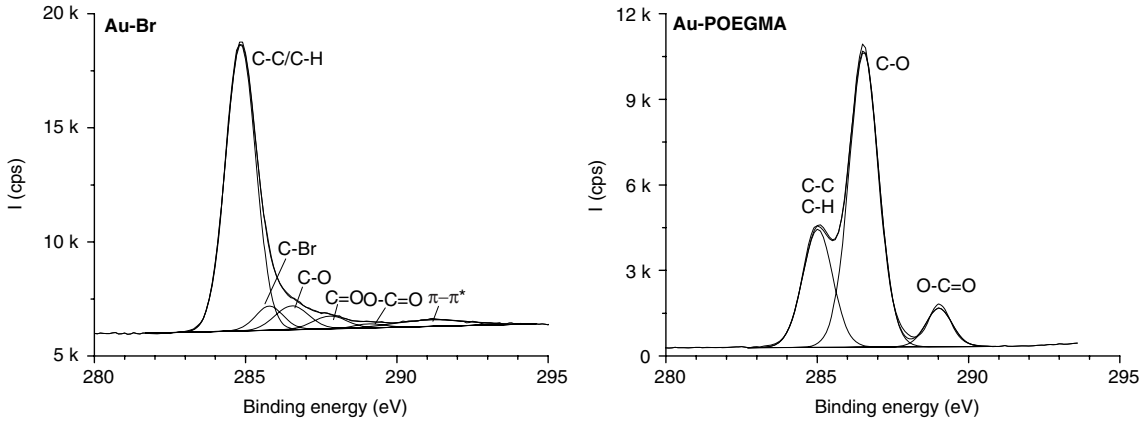

Figure 4. Peak-fitted C1s regions from Au-Br and Au-POEGMA samples.

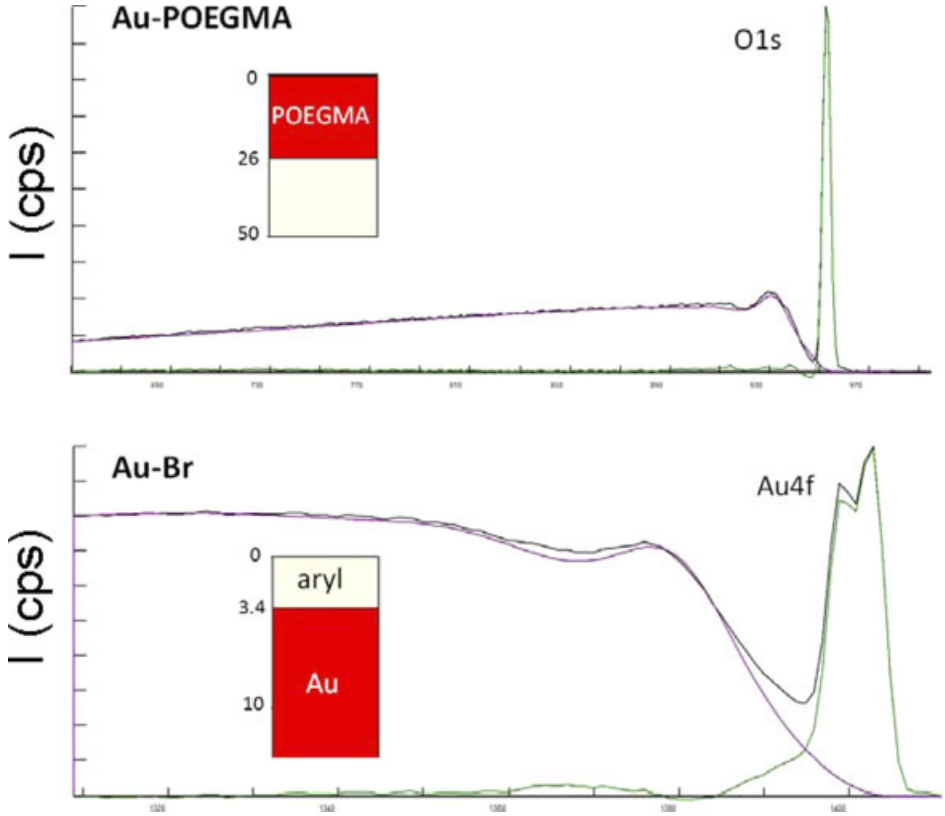

Figure 5. QUASES analysis of the O1s region from Au-POEGMA (upper panel); and the Au4f region from the Au-Br sample (lower panel). The dark regions of the boxes shown in inserts indicate the depths at which polymer (top) and gold (bottom) are found.

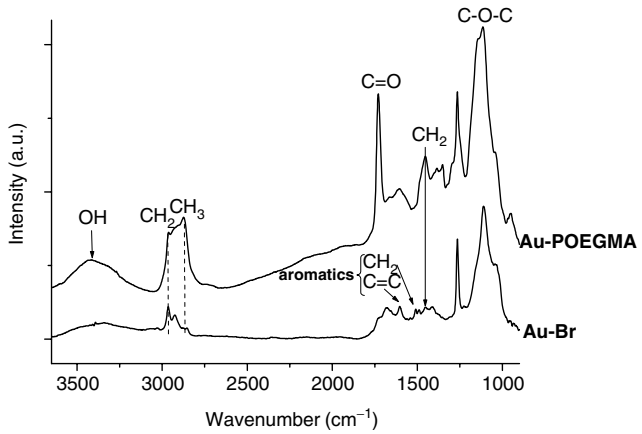

Figure 6. PMIRRAS spectra of Au-Br and Au-POEGMA.

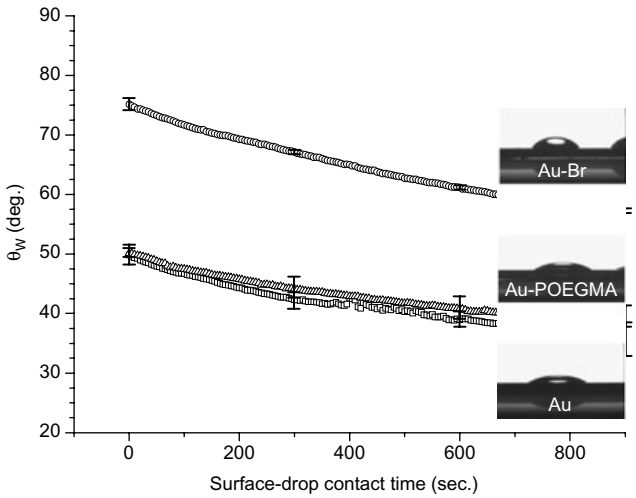

Figure 7. Plots of water contact angle (θ_w) *versus* contact time for Au, Au-Br and Au-POEGMA.

angles were recorded over a period of 15 min under controlled conditions (25 °C and stable water vapor pressure). Figure 7 shows θ_w-*vs*-time plots for Au, Au-Br and Au-POEGMA and the corresponding live video images representative of the various drop-substrate systems. Each curve corresponds to the average of the measurements of three to five liquid droplets.

The contact angles determined after 15 min of water drop-slide interactions were found to be 35.5, 57.1 and 39.5° for Au, Au-Br and Au-POEGMA, respectively. The attachment of the aryl layer leads to a gold surface that is significantly more hydrophobic. In contrast, after ATRP of OEGMA, the end-hybrid Au-POEGMA exhibits a substantial hydrophilic character with a contact angle of 39.5°. For POEGMA grafts prepared by ATRP, Brown *et al*.[23] reported advanced and receding water contact angles of 62 and 28°, respectively. These results are compatible. Indeed, Tadmor[24] showed that the equilibrium Young contact angle can readily be calculated from advancing and receding contact angles. Thus, applying the relation suggested by Tadmor to the results of Brown *et al*., one obtains a contact angle about 44.8°. Elsewhere, Chilkoti and coworkers[17] found that POEGMA films had a contact angle of 46 ± 1.0°. These values are in good agreement with the contact

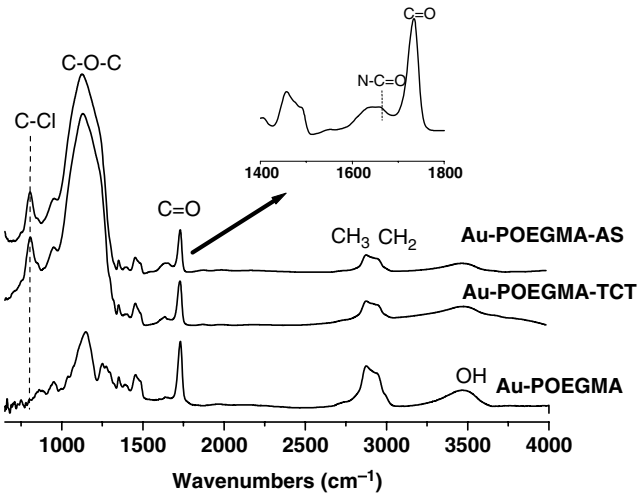

Figure 8. PM-IRRAS spectra of Au-POEGMA, Au-POEGMA-TCT and Au-POEGMA-AS.

angle we measured and which indicate a substantial hydrophilic character due to the POEGMA grafts.

Interactions of *Salmonella* with Au-POEGMA

In this section we compare bacterial adhesion on Au-POEGMA and Au-POEGMA-AS. The latter was prepared by immobilizing anti-*Salmonella* (AS) to Au-POEGMA through the TCT coupling procedure.

We first report the PM-IRRAS study of the Au-POEGMA after activation by TCT and immobilization of the AS. Figure 8 shows the PM-IRRAS spectra of TCT-modified Au-POEGMA (Au-POEGMA-TCT) and the resulting substrates Au-POEGMA-AS.

TCT is characterized by the inplane asymmetric C-Cl stretching band at 805 cm^{-1}. Note that after TCT reaction, the intensity of the OH decreased relatively to the C═O stretching band from POEGMA.

For Au-POEGMA-AS, the spectrum shows the presence of the antibody detected by the amide characteristic band centered at 1664 cm^{-1}. The insert shows a narrower region for Au-POEGMA-AS specimen. It permits to better see the changes due to the antibody.

AFM was used to image Au, Au-POEGMA and Au-POEGMA-AS plates after adsorption of bacteria from aqueous solutions. Figure 9 shows explicitly that, without any surface treatment, clean Au gets readily colonized by *Salmonella* bacteria. Here we can see that there are about 15–18 bacteria per 100 μm^2. Au-POEGMA surface is smooth (average roughness less than 2 nm) and exhibits an antifouling character due to the hydrophilic character of POEGMA. Note, however, that the insert shows a single bacterium sitting on a scratch in the polymer ultrathin film.

The other interest in using POEGMA and other hydrophilic, hydroxylated polymers is that they can be modified by proteins via several chemistry routes including the TCT option we have selected. In the present work, we have attached AS to Au-POEGMA and obtained the bioactive Au-POEGMA-AS surface. The corresponding AFM image of the plate indicates attachment of bacteria, in contrast to Au-POEGMA prior to modification. The insert shows the flagellae from a bacterium that specifically adhered to the substrate.

It is worth noting that AFM images highlight Au-POEGMA as an antifouling surface due to its hydrophilic character. Elsewhere,

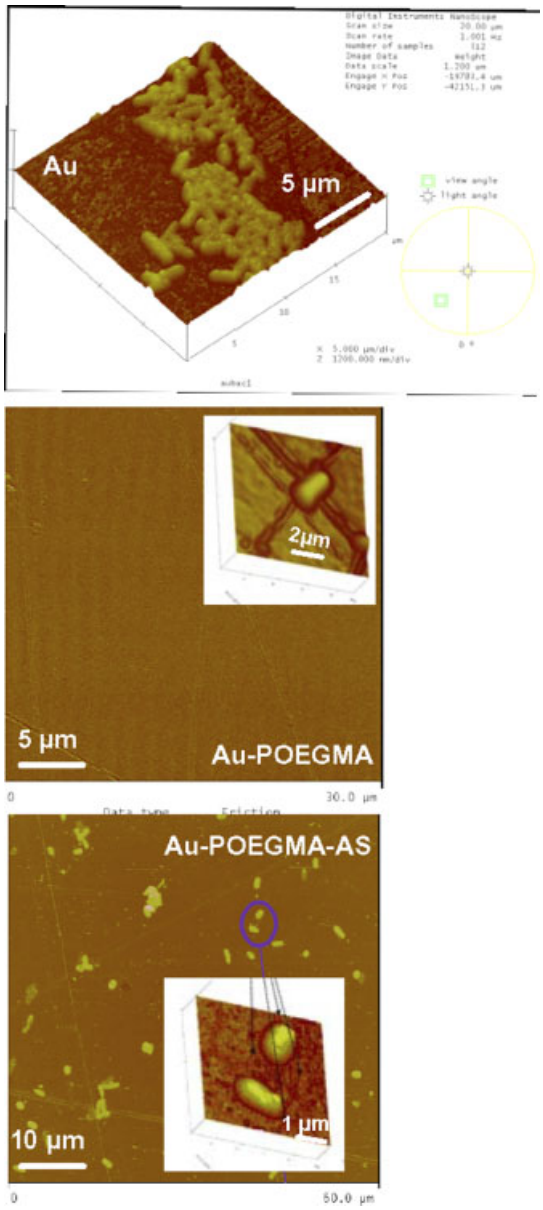

Figure 9. AFM images showing the adhesion of *Salmonella* to Au, Au-POEGMA and Au-POEGMA-AS. For Au-POEGMA; insert shows a single bacterium sitting on a scratch in the polymer layer. For Au-POEGMA-AS, the insert shows the flageallea from a bacterium that specifically adhered to the substrate.

Zhao *et al.*[25] related bacterial adhesion to the total surface-free energy computed from contact angles determined using the sessile drop method. Indeed, titanium was the most hydrophilic among a series of substrates but immobilized the highest extent of bacteria due to its relatively high surface energy. Returning to this work, uncoated gold (a high surface energy material) is slightly more hydrophilic than titanium (water contact angles are 35.5 and 42°, respectively) and was colonized by *Salmonella*. After POEGMA grafting, hydrophilicity is quasi the same but bacterial adhesion was suppressed. It is thus clear that ultrathin hydrophilic polymer grafts play an important role in bacterial adhesion provided they are densely packed and thick enough (10-nm-thick or more according to Chilkoti and coworkers[17]).

Conclusion

Gold substrate-grafted poly(oligoethylene glycol methacrylate), Au-POEGMA, were prepared by surface-confined atom transfer radical polymerization (ATRP) initiated by aryl groups from parent diazonium salts of the formulae BF_4^-, $^+N_2$-C_6H_4-$CH(CH_3)Br$. The changes in the chemical composition of gold slides were monitored by XPS, PM-IRRAS and contact angle measurements. Both survey and high resolution C1s regions of Au-POEGMA indicate a dense layer of the hydrophilic POEGMA. The hydrophilic character was emphasized by the contact angle of 39.5° much lower than 57.1° found for the initiator-modified gold slide (Au-Br).

Whilst Au immobilized bacteria nonspecifically, Au-POEGMA was found to act as an antifouling substrate. However, upon activation by anti-*Salmonella* through the trichlorotriazine coupling procedure, the antibody-functionalized Au-POEGMA slides were found to immobilize specifically *Salmonella* due to the antibody–antigen interfacial reaction operating between the immobilized antibody and the O and H antigens from the bacteria outer membrane.

This work shows conclusively that tandem aryl diazonium salt electroreduction and ATRP is an alternative, elegant protocol for the design of robust, bioactive polymer grafts.

Acknowledgements

A. Mejbri and B. Mrabet wish to thank the Faculty of Pharmacy of Monastir (Monastir, Tunisia) and the University Paris Diderot for financial support.

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