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Bacterial Resistance Control on Mineral Surfaces of Hydroxyapatite and Human Teeth via Surface Charge-Driven Antifouling Coatings

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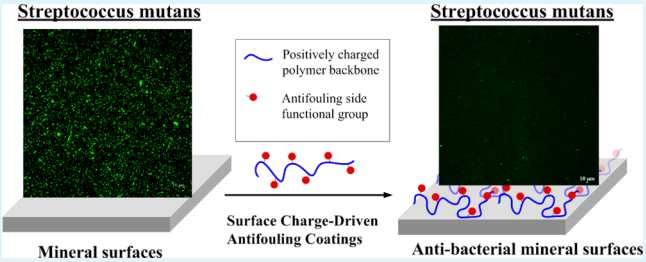
Supporting Information

ABSTRACT: This works reports a set of new functionalized polyethyleneimine (PEI) polymers, including a neutral PEGylated polymer PEI-*g*-PEGMA, a negatively charged polymer PEI-*g*-SA, and a zwitterionic polymer PEI-*g*-SBMA, and their use as antibiofouling coating agent for human teeth protection. Polymers were synthesized by Michael addition, XPS analysis revealed that each polymer could be efficiently coated onto hydroxyapatite, ceramic material used as a model tooth. Polymers carrying a negative net charge were more efficiently adsorbed, because of the establishment of electrostatic interactions with calcium ions. Protein adsorption tests revealed that two factors were important in the reduction of protein adsorption. Both the surface charge and the surface ability to bind and entrap water molecules had to be considered. PEI-*g*-SBMA, which zeta potential in PBS solution was negative, was efficient to inhibit the adsorption of BSA, a negative protein. On the other hand, it also resisted the adsorption of lysozyme, a positive protein, because zwitterionic molecules can easily entrap water and provide a very hydrophilic environment. *Streptococcus mutans* attachment tests performed unveiled that all modified polymers were efficient to resist this type of bacteria responsible for dental carries. Best results were also obtained with PEI-*g*-SBMA coating. This polymer was also shown to efficiently resist the adsorption of positively charged bacteria (*Stenotrophomonas maltophilia*). Tests performed on real human tooth showed that PEI-*g*-SBMA could inhibit up to 70% of bacteria adhesion, which constitutes a major result considering that surface of teeth is very rough, therefore physically promoting the attachment of proteins and bacteria.



KEYWORDS: *teeth protection, polyethylenimine, hydroxyapatite, PEGylated modified polymer, zwitterionic polymer, antibacterial adhesion*

INTRODUCTION

Dental caries represent a very common disease, and are often due to bacteria adhesion, such as that of *Streptococcus mutans*, and subsequent biofilm formation.[1,2] As reminded by Helfman, these bacteria, in the presence of sugars or fermentable carbohydrates, generate acids such as lactic, propionic, or acetic acid, which will attack the tooth enamel and cause tooth decay.[3] In this respect, a strategy to annihilate or at least limit tooth decay is to develop antibiofouling biomaterials applied onto the teeth. These biomaterials used in dental protection can either kill or prevent bacteria adhesion. In the first case, biomaterials possess an antibacterial activity. This function can be attributed to, for example, the introduction of quaternary ammonium salts,[4] or coating of a metal.[5] Eshed and co-workers reported recently on the use of ZnO and CuO coated on teeth model by sonochemical irradiation.[2] They managed to uniformly deposit metal coatings onto the surface of artificial teeth, resulting in a decreasing of biofilm formation of at least 70%, compared to uncoated tooth. In the second strategy, an increase in hydrophilic moieties prevents possible interactions between teeth material and proteins. Indeed, as repeated by Müller et al. (2010), the pellicle, a thin, invisible, bacteria-free protein film, is adsorbed in the oral cavity.[6] It acts as a protective laver against friction, but also favors bacteria adhesion, which outer cells are made of rather hydrophobic proteins. Prevention of bacteria attachment can be achieved by coating various types of hydrophilic materials onto the tooth, including neutral, positively- or negatively charged molecules, and zwitterions. It is a promising research direction, because it might also be applied in other fields such as the development of low-biofouling membranes by coating a hydrophilic layer onto virgin polymer membrane.[7] Finally, teeth can also be protected

Received: October 28, 2013
Accepted: February 10, 2014
Published: February 10, 2014

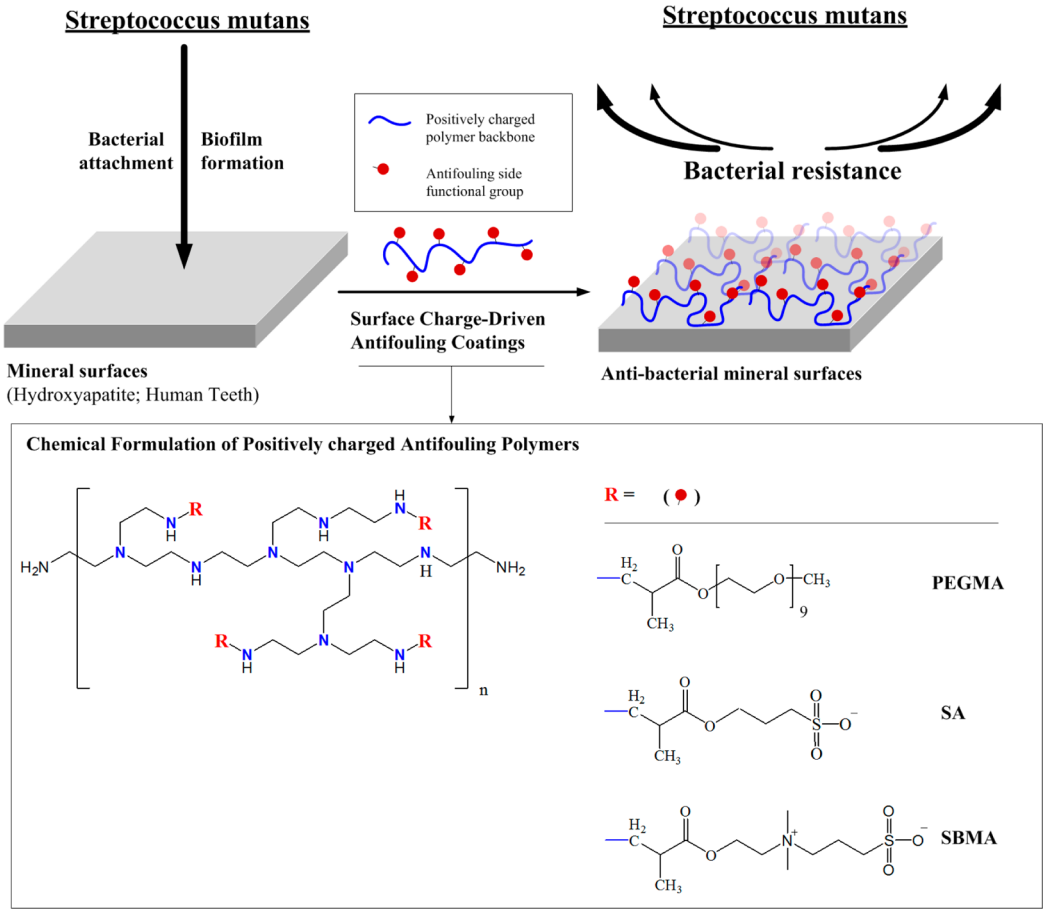


Figure 1. Schematic illustration of the preparation process of the antibacterial mineral surfaces using PEI-*g*-PEGMA, PEI-*g*-SA, and PEI-*g*-SBMA modified polymers.

from acids produced by bacteria using casein-derived phosphopeptides.[8,9] The advantage of the last approach is that these derivatives also limit dental erosion because of the consumption of acidic fruits, fruit juices, and acidic beverages.[10]

As aforementioned, the development of materials enable to prevent adhesion of bacteria is an approach investigated in various fields. Among popular antibiofouling materials, the polyethylene glycol (PEG)-systems are the most reported.[11−15] These are neutral polymers that bind water molecules via their ethylene glycol groups. Sun et al. recently used the antibiofouling property of PEG-based systems in electrochemical biosensors applied to the determination of glucose in whole blood.[11] Chen and co-workers designed and antifouling poly (ethylene oxide)-*block*-poly(g-methacryloxypropyl trimethoxysilane) coating for high quality nanocrystals used in cancer therapy and drug delivery.[12] It exhibited low nonspecific binding by macromolecules after incubation with fetal bovine serum, therefore increasing circulation time and decreasing organ toxicity. Peng and co-workers newly reported the use of PEG to modify dendrimer-entrapped gold nanoparticles for computed tomography imaging applications.[13] In another field, Peng et al. presented the grafting of poly(ether glycol) methyl ether methacrylate (PEGMA) onto polyethersulfone (PES), in order to prepare protein fouling resistant membranes.[14] Compared with PES control membrane, surface hydrophilicity of PES-*g*-PEGMA membranes was enhanced and protein adsorption significantly inhibited due to hydrogen bonding interactions between PEG segments and water. Moreover, zwitterionic molecules constitute ideal antifouling materials, by promoting the trapping of water molecules in the zwitterionic head, which leads to the inhibition of protein adsorption onto surfaces.[16−19] In addition to that, coating surfaces with zwitterion polymers has become more and more attractive because the surface charge can be controlled during their synthesis by controlling the positive groups to negative segments ratio. Consequently, antiadhesion properties toward specific charged macromolecules can be tuned.

Most bacteria, and especially those responsible for carries, are negatively charged owing to the presence of proteins, and other cell-wall components containing phosphate, carboxyl and acidic groups.[20] In this respect, an overall negatively charge surface should prevent their adhesion by promoting electrostatic repulsions. Indeed, as bacteria approach the negative charged surface, the electric double layers begin to overlap causing an electrostatic repulsive force. This offers another field of investigations, rather than only look into copolymers that would enhance hydrophilicity of surfaces, such as PEG-systems. Yet, there is a lack of investigation concerning the charge surface control of coatings applied to teeth protection. It is surely not an easy task to perform, also due to the existence of the van der Waals forces, significant attractive forces unaffected by ionic strength, operating over a small distance, and favoring adhesion of bacteria onto teeth. However, it is worth investigating this direction, since polymers or copolymers can carry significant charge density, making repulsive forces dominating and therefore preventing biofouling.

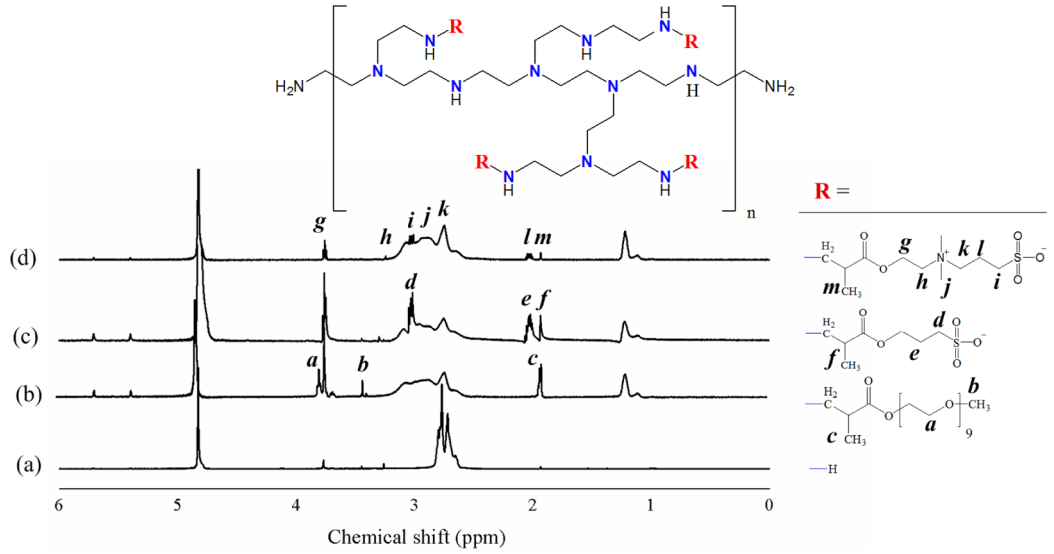


Figure 2. ^{1}H NMR spectra of (a) virgin PEI, (b) PEI-*g*-PEGMA, (c) PEI-*g*-SA, and (d) PEI-*g*-SBMA.

The scope of the present paper is to develop new functionalized modified polyethyleneimine (PEI) polymers as displayed in Figure 1, including a PEGylated PEI polymer: PEI-*g*-PEGMA, a sulfonated PEI polymer: PEI-*g*-SA, and a zwitterionic sulfobetane-grafted PEI polymer: PEI-*g*-SBMA, and investigate their efficiency to resist bacteria adhesion onto (i) tooth model made of hydroxyapatite (HA) and (ii) real human tooth. Polymer structure was assessed using ^{1}H NMR, while their global charge and swelling behavior was investigated through zeta potential and granulometry measurements, respectively. Subsequently, coating onto HA surface was performed and its efficiency assessed by XPS. Coating density as a function of polymer concentration was discussed considering the net charge carried by each polymer. Antifouling properties of new polymers regarding the adhesion of proteins (BSA and lysozyme) and bacteria (*Streptococcus mutans* and *Stenotrophomonas maltophilia*) onto HA surfaces was presented before discussing their efficiency on real human tooth.

EXPERIMENTAL SECTION

Materials. Hydroxyapaptite (HA, MW: 502 g/mol) powder, polyethylenimine (PEI, MW: 25 000 g/mol) and polyethylene glycol methyl ether methacrylate (PEGMA) monomer (MW: 475 g/mol) were purchased from Sigma-Aldrich. Sulfopropyl methacrylate (SA, MW: 246.32 g/mol) and sulfobetaine methacrylate macromonomer (SBMA, MW: 279.36 g/mol) were provided by Sigma Chemical Co and Monomer-Polymer & Dajac Laboratories, respectively. Bovine serum albumin (BSA, MW ≈ 66 000 g/mol) and lysozyme from chicken egg white (LY, MW ≈ 14 300 g/mol) were purchased from Sigma Chemical Co. Tetrahydrofuran (THF, MW: 72 g/mol), supplied by Tedia was used as a solvent without further purification. Phosphate buffer saline (PBS) was purchased from Sigma-Aldrich. Deionized water used in the experiments was purified using a Millipore water purification system with a minimum resistivity of 18.0 MΩcm.

Polymer Synthesis and Characterization. All polymers presented in this work were synthesized by Michael addition. Concerning PEI-*g*-PEGMA, 1 g of PEI was solubilized in 9 g of water, and the pH was subsequently adjusted to 11. Afterward, 3.325 g of PEGMA was added. The molar ratio of amine functions to vinyl groups was therefore controlled to 2:1. The mixture was allowed to react for 24 h at 60 °C. Then, the resulting reaction solution was precipitated into 200 mL of THF. This purification step was repeated three times, and resulted in the loss of reaction products up to 10% polymer. Finally, the polymer was dried in a vacuum oven at room temperature to yield a white powder. As for PEI-*g*-SA, 1 g of PEI was solubilized in 9 g of water, and 1.72 g of SA were added so as to control the molar ratio of amine functions to vinyl groups to 2:1. Then, the pH was adjusted to 11 and the blend was allowed to react for 24 h at 60 °C. As a suitable nonsolvent for the obtained polymer could not be found, polymer was purified by dialysis using a Spectra/Por membrane with a 6−8 kDa MWCO. Finally, polymer was freeze-dried. As for PEI-*g*-SBMA, it was synthesized by solubilizing 2 g of PEI in 8 g of water, and 1.955 g of SBMA were subsequently added (amine functions/vinyl groups molar ratio: 2:1) and reaction was performed at 90 °C for 6 h. Purification and final drying of polymer were done similarly to those of PEI-*g*-SA. The ^{1}H NMR spectra were recorded at 500 MHz with a Bruker 500 MHz NMR Spectrometer using D_2O as solvent. The polymer concentration was 10 mg/mL. The degree of modification for each functionalized polymer could be determined from results of ^{1}H NMR analysis performed with MestReC software. Further details regarding the assessment of degree of modification are provided in the Results section. Finally, hydrodynamic diameter and zeta potential of polymer solutions (5 mg/mL in PBS) were measured using a Zetasizer Nano ZS90 instrument (Malvern).

Surface Coating and Characterization. As-prepared modified polymers were coated onto HA discs, initially prepared by pressing 2 g of HA powder. HA discs were immersed into 1 mL of modified polymer PBS solution (either PEI-*g*-PEGMA, PEI-*g*-SBMA or PEI-*g*-SA) for 2 h. Concentration varied in the range 1−5 mg/mL. Then, discs were rinsed three times with PBS to remove loosely attached polymer and freeze-dried at −40 °C under a vacuum. The coating density was evaluated by UV spectrophotometry using a Biotech instrument (PowerWave XS) during the coating process. Absorbance at 220 nm of the coating bath containing the polymer was measured before immersing the HA disks and after coating. Amount of polymer adsorbed onto the surfaces was then deducted from calibration curves. Surface compositions of virgin HA and polymer-coated HA were characterized by X-ray photoelectron spectroscopy (XPS). XPS analysis was performed using a PHI Quantera SXM/Auger spectrometer with a monochromated Al KR X-ray source (1486.6 eV photons). The energy of emitted electrons was measured with a hemispherical energy analyzer at pass energies ranging from 50 to 150 eV. Data were collected at photoelectron takeoff angles of 45° with respect to the sample surface. The binding energy (BE) scale is referenced by setting the peak maximum in the C 1s spectrum to 284.6 eV. A high-resolution C1s spectrum was fitted using a Shirley background subtraction and a series of Gaussian peaks. The data analysis software was from Service Physics, Inc.

Protein Adsorption. BSA adsorption tests were performed at pH 7.4 to assess the effect of polymer-coating on resistance to biofouling

Table 1. Physical Characterization of the Polymers[a]

	degree of modification (%)						
sample ID	PEGMA	SA	SBMA	HD in PBS (nm)	ZP at pH 5 (mV)	ZP at pH 7.4 (mV)	ZP at pH 11 (mV)
PEI				3.89 ± 0.29	14.20 ± 0.95	9.89 ± 0.28	−1.59 ± 0.52
PEI-*g*-PEGMA	3.8			3.80 ± 0.53	5.29 ± 1.34	0.63 ± 1.10	−0.61 ± 1.25
PEI-*g*-SA		5		3.82 ± 0.27	8.18 ± 0.25	−3.36 ± 2.37	−12.9 ± 0.36
PEI-*g*-SBMA			3	4.06 ± 0.15	2.37 ± 0.30	−2.12 ± 1.03	−2.22 ± 1.67

[a]HD, hydrodynamic diameter; ZP, zeta potential.

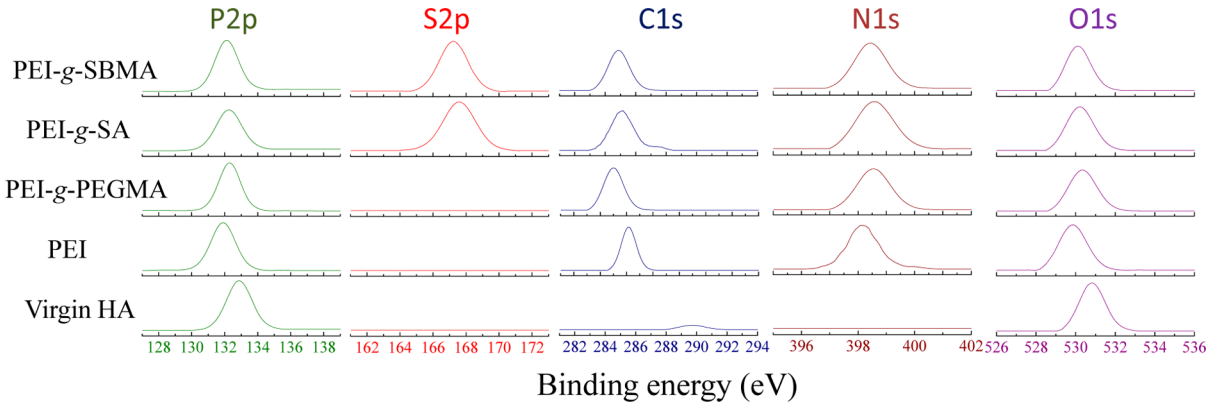


Figure 3. XPS analysis of virgin HA and polymer-coated HA surfaces with PEI, PEI-*g*-PEGMA, PEI-*g*-SA, and PEI-*g*-SBMA.

at a nanoscale. In these tests, discs were first washed in a 24-well plate with 1 mL of pure ethanol for 30-min duration. Subsequently, they were immersed in PBS for 2 h at 25 °C. Afterward, PBS was removed and disks soaked in 1 mL of 1 mg/mL BSA for 2 h at 25 °C. The absorbance at 280 nm was measured using a UV−vis spectrophotometer (PowerWave XS, Biotech). Tests were performed three times on each disc. Another protein, lysozyme, was used to evaluate resistance to protein adsorption and a similar method was employed. After immersing membranes in ethanol (30 min) and in PBS (2 h), they were incubated with 1 mL of 1 mg/mL LY for 2 h at 25 °C and absorbance measured at 280 nm.

Bacterial Attachment. *Streptococcus mutans* and *Stenotrophomonas maltophilia* were used to investigate resistance of the as-prepared coatings to bacterial adhesion. *Streptococcus mutans* was cultured in a medium containing 15.0 mg/mL tryptone, 5.0 mg/mL soytone and 15 mg/mL NaCl. *Stenotrophomonas maltophilia* was cultured in a medium containing 3.0 mg/mL beef extract and with 5.0 mg/mL peptone. These cultures were incubated at 37 °C and shaken at 100 rpm until reaching a final concentration of 10^9 cells/mL after 25 h of incubation for *S. mutans* and of 10^8 cells/mL after 22 h of incubation for *S. maltophilia*. The samples were washed 3 times with DI water in a 24-well plate. One milliliter of bacteria suspension was added to each well. Bacteria were then incubated with the samples for 24 h at 37 °C. Bacterial solution was changed every 6 h during the incubation period, to ensure that bacteria contacting the samples were in healthy state. The bacterial solution was then removed and discs washed with DI water 3 times at 37 °C. Bacteria adhering to the sample surfaces were subsequently stained with 200 μL of Live/Dead BacLight for 5 min. After washing 3 times with PBS, the samples with stained bacteria were observed using a confocal laser scanning microscope (CLSM) mounted on a resonance scanner with 200× magnification. During observation, the images were taken at λ_{ex} = 488 nm/λ_{em} = 520 nm for detection of the Live/Dead BacLight dye. This analysis was performed using a NIKON CLSM A1R instrument.

RESULTS AND DISCUSSION

Polymer Characterization. ^{1}H NMR was performed in order to ascertain the structure of modified polymers prepared in this work. Spectra and associated structures are displayed in Figure 2, as well as the assignment of the different peaks. This technique also permitted to evaluate the degree of modification for each PEI-modified polymer, and results are gathered in Table 1.

Peaks appearing at chemical shifts in the range 2−3 ppm corresponded to protons held by PEI backbone. After Michael addition of PEGMA monomer, new peaks arose on the NMR spectrum of the obtained product (Figure 2b), which could identified. Those noted a, b, and c are attributed to the protons' signal of ethylene groups, terminal methyl group of -R blocks, and methyl group in α-position of the carbonyl groups, respectively. Similarly, for PEI-*g*-SA, peaks d and e correspond to signals of protons held by carbons in α and β-positions of the sulfonate group. As for PEI-*g*-SBMA, protons noted g−m in Figure 2c could also be identified. In particular, protons h, j, and k were held by carbon atoms in α-position of the quaternary ammonium ion, while protons i and l were carried by those in α- and β-positions of the sulfonate group.

Degree of modification of PEI-*g*-PEGMA could then be determined as follows. First, the PEGMA to PEI molar ratio *r* was calculated from the following equation

$$r = \text{PEGMA/PEI} = \left(\frac{A_{H_a}}{36}\right) \Big/ \left(\frac{A_{CH_2CH_2N}}{46 \cdot 50}\right) \quad (1)$$

Where A_{H^a} refers to the area occupied by H^a protons' signal on NMR spectrum, and $A_{CH_2CH_2N}$ is that of peak corresponding to protons held by characteristic PEI functional groups (2−3 ppm). Degree of modification of PEI-*g*-PEGMA is then obtained by dividing *r* by the number of primary and secondary amines held by PEI, that is 350 in our case (since PEI is composed of 50 repeat units, each one holding 7 amine functions).

Similarly for PEI-*g*-SA and PEI-*g*-SBMA, ratios *r*′ and *r*″ were obtained, and then divided by 350 to yield the degrees of modification. Expressions for *r*′ and *r*″ are as follows

$$r' = \mathrm{SA/PEI} = \left(\frac{A_{CH_2O}}{2}\right) \Big/ \left(\frac{A_{CH_2CH_2N}}{46{\cdot}50}\right) \quad (2)$$

$$r'' = \mathrm{SBMA/PEI} = \left(\frac{A_{Hg} + A_{Hh}}{4}\right) \Big/ \left(\frac{A_{CH_2CH_2N}}{46{\cdot}50}\right) \quad (3)$$

It resulted in degree of modification relatively low (Table 1), considering the number of potential reactive sites on PEI polymer. It can be explained by the important steric hindrance of blocks added (PEGMA, SA, SBMA), preventing an optimal coverage of PEI backbone.

Moreover, the hydrodynamic diameter of all prepared polymers estimated by DLS, was in the range 3.30−4.30 nm. Therefore, the swelling behavior in PBS of all polymers was about the same. Table 1 also suggested that the dissolved polymer will show no tendency to aggregation, given the low hydrodynamic diameters measured. As for zeta potential, it was found to drop at physiological pH from a highly positive value for PEI to a slightly positive value for PEI-*g*-PEGMA, and negative values for PEI-*g*-SA and PEI-*g*-SBMA. Although PEGMA is electrically neutral, it was believed that it exerted a shadowing effect on positive charge carried by quaternary ammonium groups, contributing to lower the zeta potential. A similar effect occurred with PEI-*g*-SBMA. The negative charge carried by SA groups contributed to the final negative value of the resulting PEI-*g*-SA polymer zeta potential.

Surface Characterization of Polymer-Coated HA. XPS was used to ensure that coating was efficiently achieved. Results of this analysis are presented by Figure 3. Spectrum of control disc made of pure HA displays one major peak at BE = 530.8 eV corresponding to the binding energy of PO_4 and Ca−OH, and another peak at BE = 132.9 eV owed to phosphor atoms.[21,22] XPS spectra related to HA coated with PEI logically presents supplementary peaks related to carbon and nitrogen atoms brought by polymer at BE of 286.2 and 398.1 eV, respectively. The successful coating of PEI-*g*-SA and PEI-*g*-SBMA polymers can be confirmed by the presence of S2p peaks at BE of 167.6 and 167.3 eV, respectively. Indeed, Zhao and Shantz modified membranes using poly(sulfobetaine methacrylate) (PSBMA), and noted a peak at 164.0 eV, which they attributed to the sulfur atoms from the zwitterionic groups.[23] As for PEI-*g*-PEGMA, the observed shift of peak on C1s core level spectra toward lower binding energies may indicate the presence of supplementary C-atoms bonds owing to the presence of C−O species.

Stability of surface modification was assessed by performing further XPS analysis after immersing the different surfaces in PBS at 37 °C for 7 days. Spectra were recorded at 3, 5, and 7 days and are available in the Supporting Information section. No change was found by comparing Figure 3 to these supplementary spectra, unveiling the stability of the coating, and therefore the effectiveness of the charge-driven method.

In addition, coating density can be assessed by evaluating the weight difference per unit surface area of HA disc after immersion in polymer bath and virgin HA disc. Relative and absolute values for coating densities are plotted in Figure 4 as a function of polymer concentration in coating bath. Soaking HA disc in PEI solution does not permit an efficient coating, owing to solubility issue. Indeed, for concentrations higher than 2 wt %, PEI precipitates were formed, preventing the obtaining of efficient coating. However, all synthesized polymers are efficiently adsorbed onto HA discs. HA is a ceramic material

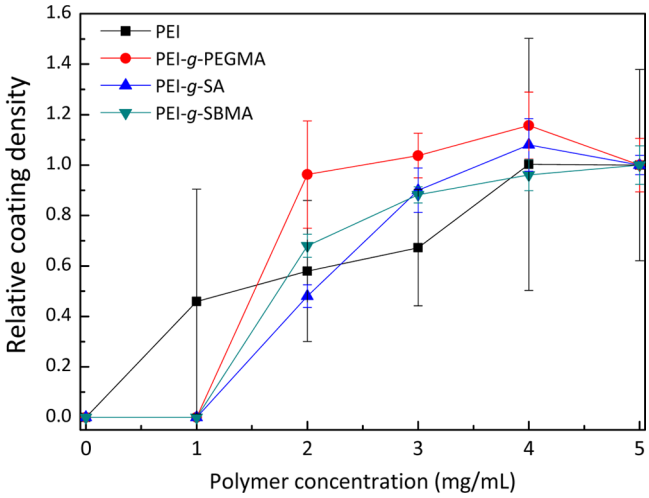


Figure 4. Relative coating density as a function of the polymer concentration in the coating bath.

composed of phosphate ions surrounded by mobile calcium ions. In this respect, local electrostatic interactions with charged polymers should be possible despite the overall zero charge of HA material, and adsorption of these polymers materials facilitated compared to neutral polymers. PEI-*g*-SBMA carries both positive charges (via its quaternary amines) and negative ones (sulfonate groups), but zeta potential measurements indicate that there is an overall excess of negative functions. Figure 4 shows that its adsorption onto HA discs is the most efficient. It is assumed that positive counterions surrounding phosphate groups of HA were readily bonded to the excess of sulfonate groups, hence contributing to favorable interactions between HA and the polymer. Necessarily, coating density of PEI-*g*-SA was expected to be high as well, considering the negative zeta potential and the result obtained with PEI-*g*-SBMA. Finally, PEI-*g*-PEGMA is a neutral amphiphilic polymer, which is confirmed by the zeta potential values varying between negative and positive values around 0 mV. It is therefore not likely to establish electrostatic-like interactions. In this respect, its adsorption onto HA discs is not easily achieved, but still possible. Figure 4 also supports the assumption of efficient coating of PEI-*g*-PEGMA onto HA disks drawn from the analysis of XPS data. To conclude, one should also note that a plateau is reached from a concentration of about 4 mg/mL, whatever the polymer, corresponding to the saturation of the HA surface by polymer molecules.

Protein Resistance of Polymer-Coated HA Surfaces. If model tooth, that is, HA surface, can effectively resist the adsorption of proteins, bacterial attachment will be likely reduced as well.[6] In this work, BSA and lysozyme were tested to assess the resistance of coated surfaces to protein adsorption. As for BSA adsorption, results presented in Figure 5 evidence that all polymers permitted to reduce interactions between HA surface and protein. However, coating with PEI was not efficient at all, and even promoted the adsorption of BSA owing to its positive nature favoring electrostatic repulsions. On the other hand, the three polymers were very efficient to reduce BSA adsorption from a 4 mg/mL concentration, which could be further correlated to the coating density results earlier presented in Figure 4 showing that a dense coating was reached from a 4 mg/mL polymer concentration. The effectiveness of polymers was related to their nature. Concerning PEI-*g*-

dx.doi.org/10.1021/am404780w | ACS Appl. Mater. Interfaces 2014, 6, 3201−3210

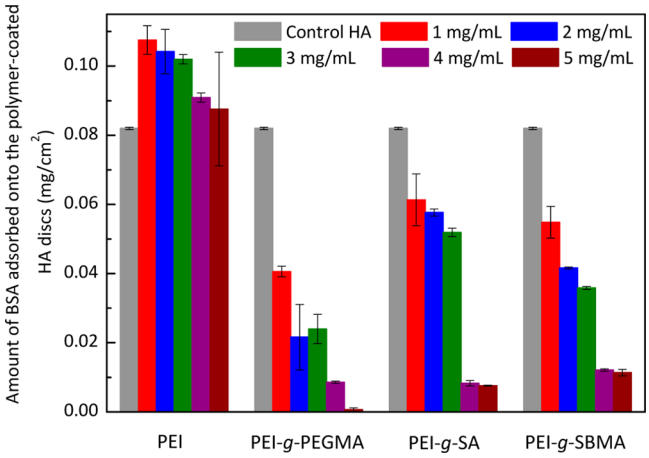


Figure 5. BSA adsorption onto the polymer-coated HA discs as a function of the nature of coating agent and of its concentration.

PEGMA, PEG-like brushes have been shown to be appropriate antibiofouling agents in many situations.[11−15,24−26] Indeed, they favor trapping of water molecules, and thus increase the wettability of surfaces. Therefore, protein adsorption is minimized since hydrophobic−hydrophobic interactions between the protein and the surface can hardly be established. As for PEI-*g*-SA, it is a negatively charged polymer (Table 1). In this respect, repulsive interactions with negatively charged BSA occur, minimizing its adsorption. Finally, PEI-*g*-SBMA is also negatively charged but its zwitterionic nature also makes it an outstanding water-trapping polymer[16−19,27] allowing it to considerably decrease protein adsorption.

Results concerning LY, a positively charged protein in the conditions of the tests, are displayed in Figure 6. PEI-*b*-SA was

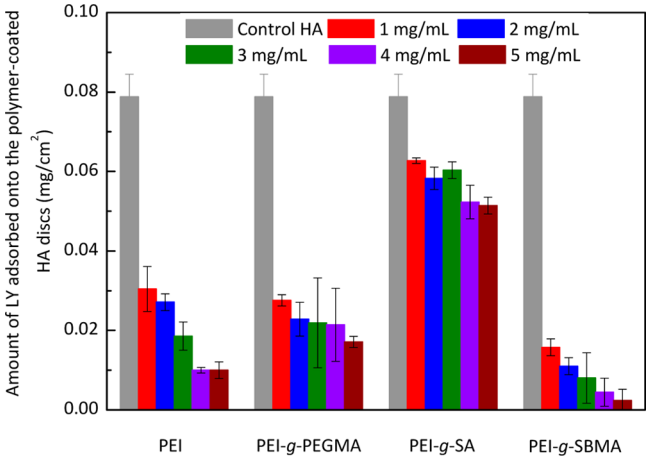


Figure 6. LY adsorption onto the polymer-coated HA discs as a function of the nature of coating agent and of its concentration.

clearly not efficient to inhibit its adsorption, because this polymer is negatively charged, and therefore exerted attractive electrostatic interactions toward LY. On the contrary, there were repulsive interactions between PEI, positively charged, and LY. In addition, the neutral polymer (PEI-*g*-PEGMA) and the zwitterionic one (PEI-*g*-SBMA) permitted to decrease LY adsorption up to 75% and 90% of the limitation of uncoated HA surface, respectively. Indeed, and as for results concerning BSA adsorption, PEGylated brushes as well as zwitterionic heads provide optimum environment for water-trapping, therefore minimizing protein adsorption. It is worth noting that despite its overall negative charge, PEI-*g*-SBMA remained very efficient to inhibit LY adsorption. It highlights its outstanding ability to entrap water, leading to a very hydrophilic surface, and preventing adsorption of proteins even in the presence of attractive forces. In conclusion, results of both Figures 5 and 6 demonstrate the efficiency of PEI-*g*-PEGMA and PEI-*g*-SBMA polymers to resist protein adsorption, whatever the nature of the protein, which supports the results of other studies in various fields showing the efficiency of PEGylated derivatives as well as that of zwitterionic structures to resist fouling due to nonspecific protein adhesion.[28−31]

Resistance to Bacterial Attachment of Polymer-Coated HA and Human Tooth Surfaces. The main goal of this research work was to design a coating material that could be able to resist the adhesion of *Streptococcus mutans*, a gram-positive and major type of bacteria involved in caries formation.[2,32,33] Decreasing its adhesion will permit to minimize biofilm formation and subsequent attack of teeth-making tissues. In this respect, tests were conducted aiming at immersing into bacteria solution HA surfaces coated with the different polymers prepared in this work, and qualitatively and quantitatively evaluating the number of colonies attaching onto the surfaces. Images obtained by confocal microscopy are displayed in Figure 7. Result related to HA control showed the need for recovering this biocompatible ceramic with an appropriate antibiofouling agent, for future applications in dentistry. PEI alone already permitted to decrease the attachment of SM, because fewer green stains were visible onto the surfaces of PEI-coated HA, due to its hydrophilic nature. But because of electrically positive charges carried by PEI, numerous negatively charged bacteria still attached the surface, since electrostatic interactions favored bacteria adhesion. On the other hand, the three subsequent images related to PEI-*g*-PEGMA-, PEI-*g*-SA-, and PEI-*g*-SBMA-coated HA discs highlighted the efficiency of the various polymers to resist SM adhesion. Indeed, these polymers provide hydrophilic protective environment to HA discs, therefore preventing their colonization by bacteria.

Besides, the efficiency of the three polymers to resist bacteria adhesion can be ranked. From the qualitative data, PEI-*g*-SBMA appeared to be the most efficient polymer. The dipole formed by the zwitterionic structure was more efficient to inhibit biofouling at a macro-scale than that formed by PEGMA hydrophilic segments. The effect of similar zwitterionic structures on inhibition of bacteria adhesion has been reported in studies dealing with the design of antibiofouling surfaces for instance.[6,34] Hence, Lalani and Liu designed electrospun zwitterionic poly(sulfobetaine methacrylate) fibers, that showed efficient resistance to adhesion of both a gram-negative bacteria (*Pseudomonas aeruginosa*) and a gram-positive one (*Staphylococcus epidermidis*) after 24 h incubation.[34] PEGylated materials have also been regularly cited in the design of surfaces resisting bacteria adhesion over the last 15 years.[25,35−37] Also, when this kind of material is reported as being an appropriated antiprotein adhesion agent, it usually exhibits similar behavior toward bacterial attachment, because similar phenomenon is involved. Indeed, PEG systems are popular antibiofouling materials because they permit a decrease in interfacial free energy, therefore allowing a reduction of both protein adsorption and bacteria attachment.[37]

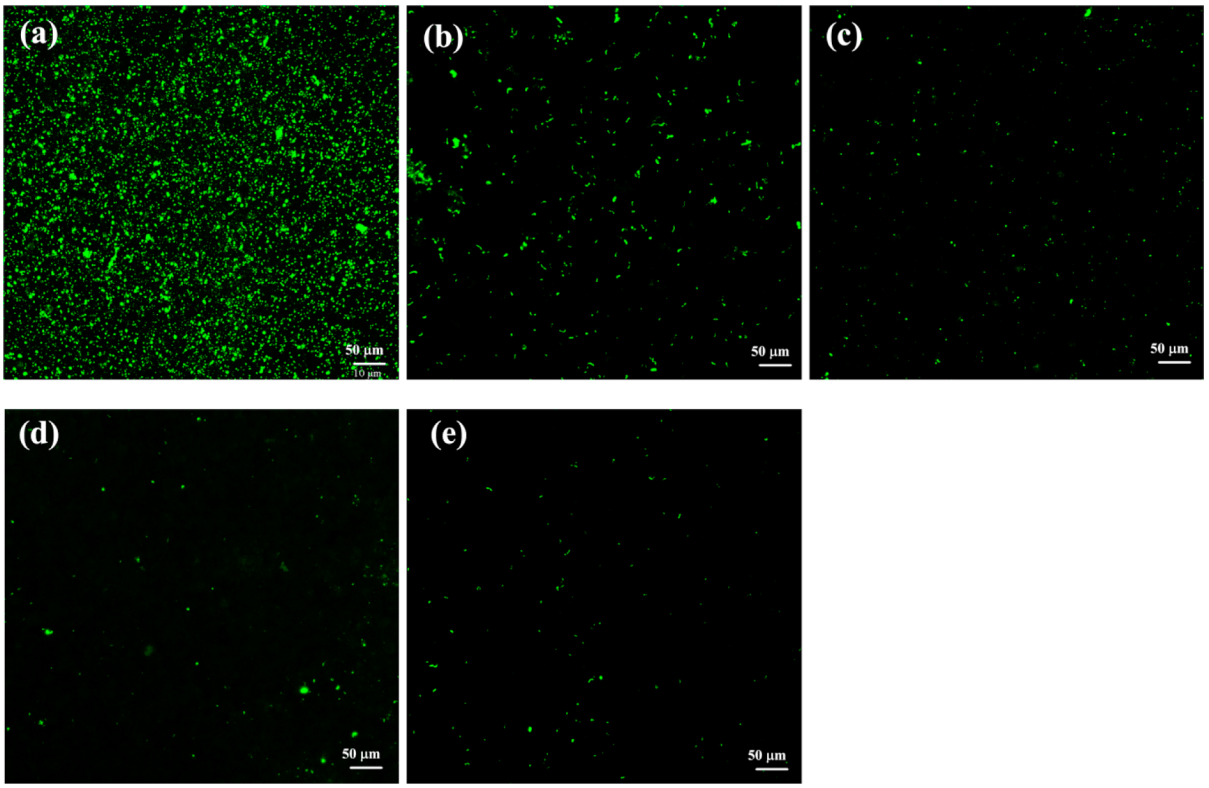


Figure 7. Qualitative results of attachment tests of *Streptococcus mutans* (a) virgin HA surface, (b) PEI-coated HA surface, (c) PEI-*g*-PEGMA-coated HA surface, (d) PEI-*g*-SA-coated HA surface, and (e) PEI-*g*-SBMA-coated HA surface. Concentration of the polymer in coating bath was 5 mg/mL. Scale bar is 50 μm.

As for the relative efficiency of PEI-*g*-PEGMA compared to that of PEI-*g*-SA, it cannot be assessed from Figure 7, but the quantitative analysis showed by Figure 8 highlights that PEI-*g*-SA is a bit more effective. This is also explained by the negative charges carried by the polymer, favoring repulsion of bacteria whose outer cell walls are negatively charged as well.

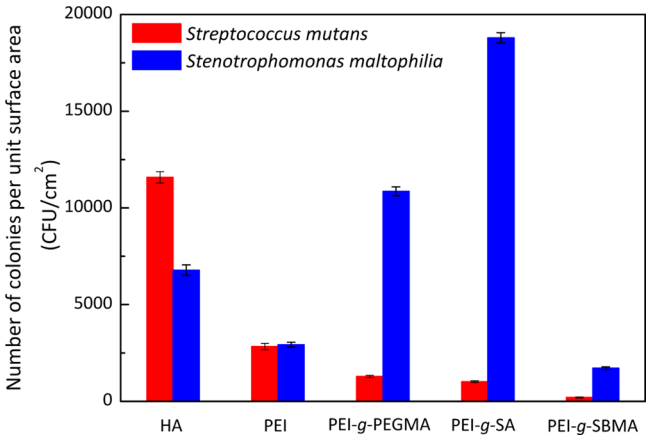


Figure 8. Quantitative analysis of the changes in *Streptococcus mutans* and *Stenotrophomonas maltophilia* attachment to the surfaces of the virgin HA discs and the polymer-coated discs.

Another bacterium, carrying positive charges, was used in adhesion tests to further demonstrate the important effect of electrostatic interactions in bacteria adhesion. Qualitative results concerning attachment tests of *Stenotrophomonas maltophilia* a gram-negative bacillus, are displayed in Figure 9. Again, incubation tests were carried out for 24 h, to maximize potential interactions between surfaces and micro-organisms. Totally different trend from that obtained with *S. mutans* was found. Indeed, virgin HA as well as HA coated with PEI do not tend to attach this bacillus. Indeed, electrostatic repulsive interactions occur with the calcium ions of HA surface or the positively charged PEI. On the other hand, surfaces coated with PEI-*g*-PEGMA and PEI-*g*-SA polymers do not resist bacterial attachment. Only PEI-*g*-SBMA coating presents quite good anti-*S. maltophilia* attachment, because of its zwitterionic and hydrophilic nature aforementioned, but some bacteria are still present because of the net negative charge carried, as well as to the van der Waals attractive forces (Figure 8).

The important conclusion herein is that a coating that satisfies the two following conditions. (i) charge opposite to that of bacteria cell-wall and (ii) ability to entrap water molecules to provide a very hydrophilic environment. will have its resistance to bacteria adhesion optimized.

Finally, investigations on *S. mutans* attachment were carried out with teeth coated with PEI or with a modified polymer synthesized in this work (Figures 10). Note that bacterial adhesion does not only depend on the chemical nature of the surface, but also on its structure. Indeed, rough surfaces will offer more adsorption sites to bacteria than smoother ones. Moreover, as pointed out by Esched and co-workers, coating teeth is challenging owing to their surface topography and hardness.[2] In addition, roughness affects the noise on images obtained by confocal microscopy, so that one has to be careful when analyzing the results, in order to distinguish the stains due to bacteria (diameter 2−6 μm) from those related to asperities (larger stains).

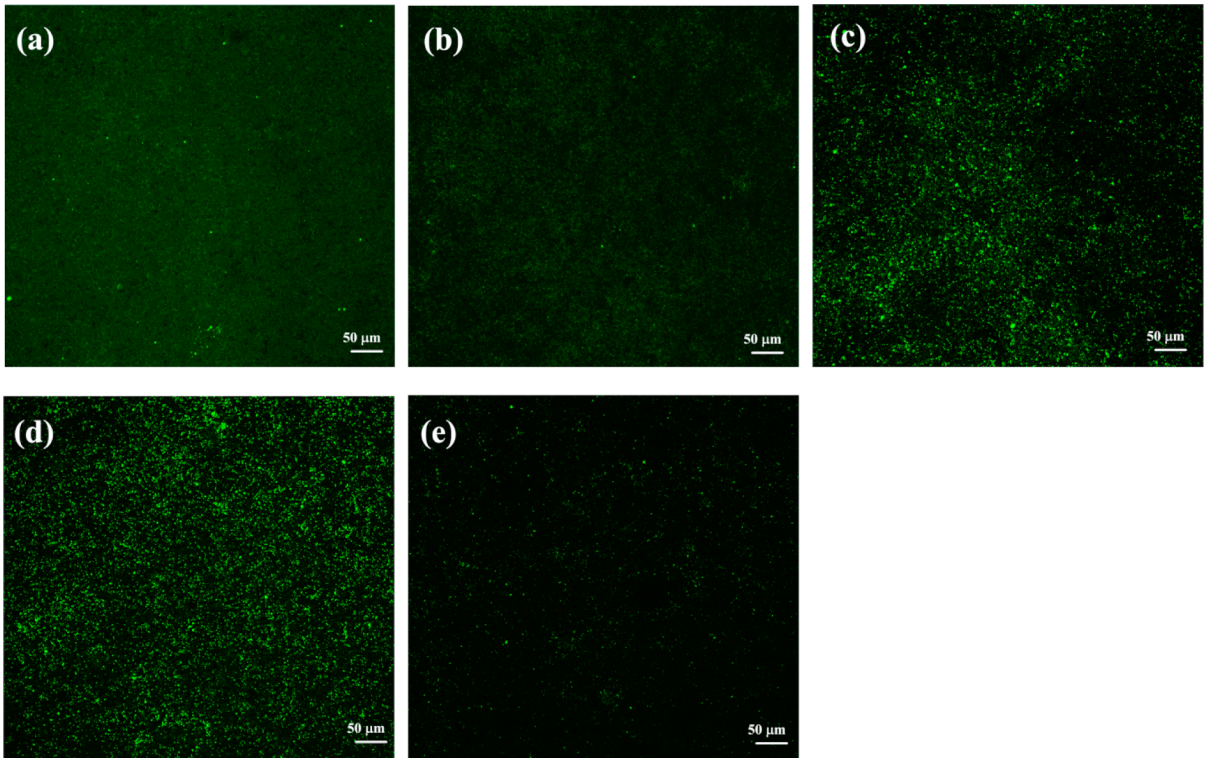


Figure 9. Qualitative results of attachment tests of *Stenotrophomonas maltophilia* onto (a) virgin HA surface, (b) PEI-coated HA surface, (c) PEI-*g*-PEGMA-coated HA surface, (d) PEI-*g*-SA-coated HA surface, and (e) PEI-*g*-SBMA-coated HA surface. Concentration of the polymer in coating bath was 5 mg/mL. Scale bar is 50 μm.

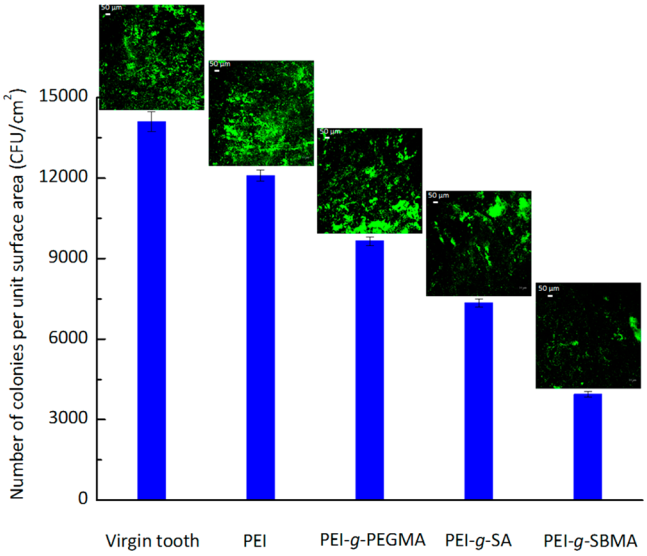


Figure 10. Qualitative and quantitative analysis of the changes in *Streptococcus mutans* attachment to the surfaces of (a) virgin HA surface, (b) PEI-coated tooth surface, (c) PEI-*g*-PEGMA-coated tooth surface, (d) PEI-*g*-SA-coated tooth surface, and (e) PEI-*g*-SBMA-coated tooth surface. Concentration of the polymer in coating bath was 5 mg/mL..

As expected, qualitative and quantitative results showed that uncoated tooth was very sensitive to the attachment of *S. mutans*. Despite a difficult analysis, coating efficiency to resist bacteria adhesion could still be classified in the following order: PEI < PEI-*g*-PEGMA < PEI-*g*-SA < PEI-*g*-SBMA, where the symbol "<" stands for "less efficient". This ranking also corresponded to the trend obtained and discussed in Figure 7 so that similar chemical mechanisms responsible for bacteria adhesion were involved. Necessarily, more bacteria were found onto the tooth, when comparing quantitative data of Figure 10 to those of Figure 8, because tooth is rougher than the HA model surface, so that entrapment of bacteria because of physical effects occurred onto the teeth as well. Still, less than 4000 CFU/cm^2 were counted onto the tooth coated with PEI-*g*-SBMA, that is, bacteria adhesion was reduced to more than 70% the limitation of the virgin tooth, which evidenced the very good antibiofouling properties of this new polymer.

CONCLUSIONS

In this study, the focus was laid on the bacterial resistance control on hydroxyapatite and human teeth via surface charge-driven antifouling coatings. Three different new polymers were synthesized including PEI-*g*-PEGMA, a neutral polymer, PEI-*g*-SA, a negatively charged polymer and PEI-*g*-SBMA, a zwitterionic polymer carrying a net negative charge.

After assessing the structures by ^{1}H NMR, and demonstrating an efficient coating via XPS analysis, it was shown that coating density onto hydroxyapatite surfaces depended on the charge carried by the polymer. Indeed, negatively charged polymers (PEI-*g*-SBMA and PEI-*g*-SA) were preferentially adsorbed, because of the presence of positive counterions onto HA surfaces. Yet, neutral polymer such as PEI-*g*-PEGMA could still be readily adsorbed onto HA surfaces, because of its hydrophilic nature.

Polymer coatings were found to be very efficient to resist BSA adsorption because of their hydrophilic segments, coupled to the global negative charge in the specific case of PEI-*g*-SA and PEI-*g*-SBMA. On the other hand, PEI-*g*-SA could not effectively resist LY adsorption, a positive protein. It was

believed that its hydrophilic nature could not balance the important electrostatic attractive forces exerted between the polymer and the protein. PEI-*g*-SBMA was still efficient, because of its zwitterionic nature favoring water molecules trapping.

Bacteria attachment tests also revealed the concomitant importance of the charge of the polymer and its ability to bind water. The best results in terms of resistance to bacteria adhesion were obtained with PEI-*g*-SBMA polymer. Whether coated onto HA surface or real human tooth, it presented outstanding antifouling property. Indeed, it possesses a great capacity to bind water. Moreover, in this study, this zwitterionic polymer was carrying an overall negative charge which further enhanced the resistance to *Streptococcus mutans*, a major bacteria negatively charged involved in carries.

On-going studies conducted onto real human teeth coated with PEI-*g*-SBMA polymer concern their efficiency to resist the bacteria adhesion and biofilm formation when incubating for several days in the bacterial bath. In addition, the effect of electric charge carried by the polymer on bacteria attachment resistance is currently being further investigated.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

XPS tests performed after immersing the coated surfaces for 3, 5, and 7 days in PBS at 37 °C, which highlight the stability of the coatings. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Center-of-Excellence (COE) Program on Membrane Technology from the Ministry of Education (MOE), R.O.C., the project of Outstanding Professor Research Program in the Chung Yuan Christian University, Taiwan (CYCU-00RD-RA002-11757), and the National Science Council (NSC 100-2628-E-033-001-MY3 and NSC 102-2221-E-033-009-MY3) for their financial support.

■ REFERENCES

(1) Koga, T.; Oho, T.; Shimazaki, Y.; Nakano, Y. Immunization Against Dental Caries. *Vaccine* **2002**, *20*, 2027−2044.

(2) Eshed, M.; Lellouche, J.; Matalon, S.; Gedanken, A.; Banin, E. Sonochemical Coatings of ZnO and CuO Nanoparticles Inhibit Streptococcus mutans Biofilm Formation on Teeth Model. *Langmuir* **2012**, *28*, 12288−12295.

(3) Helfman, M. Chemical Aspects of Dentistry. *J. Chem. Educ.* **1982**, *59*, 666−668.

(4) Kenawy, E. R.; Worley, S. D.; Broughton, R. The Chemistry and Applications of Antimicrobial Polymers: A State-of-the-Art Review. *Biomacromolecules* **2007**, *8*, 1359−1384.

(5) Phan, T. N.; Buckner, T.; Sheng, J.; Baldeck, J. D.; Marquis, R. E. Physiologic Actions of Zinc Related to Inhibition of Acid and Alkali Production by Oral Streptococci in Suspensions and Biofilms. *Oral Microbiol. Immunol.* **2004**, *19*, 31−38.

(6) Müller, C.; Lüders, A.; Hoth-Hannig, W.; Hannig, M.; Ziegler, C. Initial Bioadhesion on Dental Materials as a Function of Contact Time, pH, Surface Wettability, and Isoelectric Point. *Langmuir* **2010**, *26*, 4136−4141.

(7) Chang, Y.; Chang, W. J.; Shih, Y. J.; Wei, T. C.; Hsiue, G. H. Zwitterionic Sulfobetaine-Grafted Poly(vinylidene fluoride) Membrane with Highly Effective Blood Compatibility via Atmospheric Plasma-Induced Surface Copolymerization. *ACS Appl. Mater. Interfaces* **2011**, *3*, 1228−1237.

(8) Kanekanian, A. D.; Williams, R. J. H.; Brownsell, V. L.; Andrews, A. T. Caseinophosphopeptides and Dental Protection Concentration and pH Studies. *Food Chem.* **2008**, *107*, 1015−1021.

(9) Setarehnejad, A.; Kanekanian, A.; Tatham, A.; Abedi, A. H. The Protective Effect of Caseinomacropeptide Against Dental Erosion Using Hydroxyapatite as a Model System. *Int. Dairy J.* **2010**, *20*, 652−656.

(10) Amaechi, B. T.; Higham, S. M. Dental Erosion: Possible Approaches to Prevention and Control. *J. Dent.* **2005**, *33*, 243−252.

(11) Sun, C.; Miao, J.; Yan, J.; Yang, K.; Mao, C.; Ju, J.; Shen, J. Applications of Antibiofouling PEG-Coating in Electrochemical Biosensors for Determination of Glucose in Whole Blood. *Electrochim. Acta* **2013**, *89*, 549−554.

(12) Chen, H.; Wang, L.; Yeh, J.; Wu, X.; Cao, Z.; Wang, Y. A.; Zhang, M.; Yang, L.; Mao, H. Reducing Non-specific Binding and Uptake of Nanoparticles and Improving Cell Targeting with an Antifouling PEO-b-P*γ*MPS Copolymer Coating. *Biomaterials* **2010**, *31*, 5397−5407.

(13) Peng, C.; Zheng, L.; Chen, Q.; Shen, M.; Guo, R.; Wang, H.; Cao, X.; Zhang, G.; Shi, X. PEGylated Dendrimer-Entrapped Gold Nanoparticles for in Vivo Blood Pool and Tumor Imaging by Computed Tomography. *Biomaterials* **2012**, *33*, 1107−1119.

(14) Peng, J.; Su, Y.; Shi, Q.; Chen, W.; Jiang, Z. Protein Fouling Resistant Membrane Prepared by Amphiphilic Pegylated Polyethersulfone. *Bioresour. Technol.* **2011**, *102*, 2289−2295.

(15) Eshet, I.; Freger, V.; Kasher, R.; Herzberg, M.; Lei, J.; Ulbricht, M. Chemical and Physical Factors in Design of Antibiofouling Polymer Coatings. *Biomacromolecules* **2012**, *12*, 2681−2685.

(16) Yang, R.; Xu, J.; Ozaydin-Ince, G.; Wong, S. Y.; Gleason, K. K. Surface-Tethered Zwitterionic Ultrathin Antifouling Coatings on Reverse Osmosis Membranes by Initiated Chemical Vapor Deposition. *Chem. Mater.* **2011**, *23*, 1263−1272.

(17) Chang, Y.; Liao, S. C.; Higuchi, A.; Ruaan, R. C.; Chu, C. W.; Chen, W. Y. A Highly Stable Nonbiofouling Surface with Well-Packed Grafted Zwitterionic Polysulfobetaine for Plasma Protein Repulsion. *Langmuir* **2008**, *24*, 5453−5458.

(18) Liu, Q.; Singh, A.; Liu, L. Amino Acid-Based Zwitterionic Poly(serine methacrylate) as an Antifouling Material. *Biomacromolecules* **2013**, *14*, 226−231.

(19) Wang, T.; Wang, Y. Q.; Su, Y. L.; Jiang, Z. Y. Antifouling Ultrafiltration Membrane Composed of Polyethersulfone and Sulfobetaine Copolymer. *J. Membr. Sci.* **2006**, *280*, 343−350.

(20) Lichter, J. A.; Van Vliet, K. J.; Rubner, M. F. Design of Antibacterial Surfaces and Interfaces: Polyelectrolyte Multilayers as a Multifunctional Platform. *Macromolecules* **2009**, *42*, 8573−8586.

(21) Santos, J. D.; Jha, L. J.; Monteiro, F. J. In Vitro Calcium Phosphate Formation on SiO2-Na2O-CaO-P2O5 Glass Reinforced Hydroxyapatite Composite: A Study by XPS Analysis. *J. Mater. Sci.: Mater. Med.* **1996**, *7*, 181−185.

(22) Murakami, A.; Arimoto, T.; Suzuki, D.; Iwai-Yoshida, M.; Otsuka, F.; Shibata, Y.; Igarashi, T.; Kamijo, R.; Miyazaki, T. Antimicrobial and Osteogenic Properties of a Hydrophilic-Modified Nanoscale Hydroxyapatite Coating on Titanium. *Nanomedicine: NBM* **2012**, *8*, 374−382.

(23) Zhao, Y. H.; Shantz, D. F. Modified Anopore Hybrid Membranes For the Microfiltration of Cellulose Acid Hydrolysis Mixtures. *J. Membr. Sci.* **2011**, *377*, 99−109.
(24) Higuchi, A.; Sugiyama, K.; Yoon, B. O.; Sakurai, M.; Hara, M.; Sumita, M.; Sugawara, S. I.; Shirai, T. Serum Protein Adsorption and Platelet Adhesion on Pluronic-Adsorbed Polysulfone Membranes. *Biomaterials* **2003**, *24*, 3235−3245.
(25) Ding, X.; Yang, C.; Lim, T. P.; Hsu, L. Y.; Engler, A. C.; Hedrick, J. L.; Yang, Y. Y. Antibacterial and Antifouling Catheter Coatings Using Surface Grafted PEG-b-cationic Polycarbonate Diblock Copolymers. *Biomaterials* **2012**, *33*, 6593−6603.
(26) Chiag, Y. C.; Chang, Y.; Chen, W. Y.; Ruaan, R. C. Biofouling Resistance of Ultrafiltration Membranes Controlled by Surface Self-Assembled Coating with PEGylated Copolymers. *Langmuir* **2012**, *28*, 1399−1407.
(27) Tu, Q.; Wang, J. C.; Liu, R.; Zhang, Y.; Xu, J.; Liu, J.; Yuan, M. S.; Liu, W.; Wang, J. Synthesis of Polyethylene Glycol- and Sulfobetaine-Conjugated Zwitterionic Poly(l-lactide) and Assay of its Antifouling Properties. *Colloids. Surf., B* **2013**, *102*, 331−340.
(28) Chen, H.; Yuan, L.; Song, W.; Wu, Z.; Li, D. Biocompatible polymer materials: Role of Protein−Surface interactions. *Prog. Polym. Sci.* **2008**, *33*, 1059−1087.
(29) Dimitriu, M. D.; Zhou, Z.; Yoo, H. S.; Killops, K. L.; Finlay, J. A.; Cone, G.; Sundaram, H. S.; Lynd, N. A.; Barteau, K. P.; Campos, L. M.; Fisher, D. A.; Callow, M. E.; Callow, J. E.; Ober, C. K.; Hawker, C. J.; Kramer, E. J. A General Approach to Controlling the Surface Composition of Poly(ethylene oxide)-Based Block Copolymers for Antifouling Coatings. *Langmuir* **2011**, *27*, 13762−13772.
(30) Yang, R.; Gleason, K. Ultrathin Antifouling Coatings with Stable Surface Zwitterionic Functionality by Initiated Chemical Vapor Deposition (iCVD). *Langmuir* **2012**, *28*, 12266−12274.
(31) Binazadeh, M.; Kabiri, M.; Unsworth, L. D. In *Proteins at Interfaces III: State of the Art*, Horbett, T., Brash, J. L., Norde, W., Eds.; ACS Symposium Series; American Chemical Society: Washington, D.C., 2012; Chapter 28, pp 621−643.
(32) Daglia, M.; Tarsi, R.; Papetti, A.; Grisoli, P.; Dacarro, C.; Pruzzo, C.; Gazzani, G. Antiadhesive Effect of Green and Roasted Coffee on *Streptococcus mutans*' Adhesive Properties on Saliva-Coated Hydroxyapatite Beads. *J. Agric. Food Chem.* **2002**, *50*, 1225−1229.
(33) Stauder, M.; Papetti, A.; Mascherpa, D.; Schito, A. M.; Gazzani, G.; Pruzzo, C.; Daglia, M. Antiadhesion and Antibiofilm Activities of High Molecular Weight Coffee Components against *Streptococcus mutans*. *J. Agric. Food Chem.* **2010**, *58*, 11662−11666.
(34) Lalani, R.; Liu, L. Electrospun Zwitterionic Poly(Sulfobetaine Methacrylate) for Nonadherent, Superabsorbent, and Antimicrobial Wound Dressing Applications. *Biomacromolecules* **2012**, *13*, 1853−1863.
(35) Park, K. D.; Kim, Y. S.; Han, D. K.; Kim, Y. H.; Lee, E. H.; Suh, H.; Choi, K. S. Bacterial Adhesion on PEG Modified Polyurethane Surfaces. *Biomaterials* **1998**, *19*, 851−859.
(36) Ekblad, T.; Bergström, G.; Ederth, T.; Conlan, S. L.; Mutton, R.; Clare, A. S.; Wang, S.; Liu, Y.; Zhao, Q.; D'Souza, F.; Donnely, G. T.; Willemsen, P. R.; Pettitt, M. E.; Callow, M. E.; Callow, J. A.; Liedberg, B. Poly(ethylene glycol)-Containing Hydrogel Surfaces for Antifouling Applications in Marine and Freshwater Environments. *Biomacromolecules* **2008**, *9*, 2775−2783.
(37) Rana, D.; Matsuura, T. Surface Modifications for Antifouling Membranes. *Chem. Rev.* **2010**, *110*, 2448−2471.