Antibacterial and Antifouling Polymer Brushes Incorporating Antimicrobial Peptide

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Surface-initiated atom transfer radical polymerization (ATRP) has been used to prepare antifouling copolymer brushes based on 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) and hydroxyl-terminated oligo(ethylene glycol) methacrylate (HOEGMA). The amount of hydroxyl reactive groups incorporated into the brushes was varied by changing the composition of the monomer mixture. These coatings were subsequently functionalized by a natural antibacterial peptide, magainin I, via an oriented chemical grafting on hydroxyl groups, which maintains the activity of the peptide. The antibacterial activity of the functionalized brushes was successfully tested against two different strains of gram-positive bacteria.

INTRODUCTION

The adhesion of bacteria on the surfaces of materials is of crucial importance in diverse fields such as ship hull fouling, contamination of medical devices, industrial cooling water systems, and food processing equipment (*1*−*3*). Once adhered on a solid surface, bacteria form colonies and subsequently biofilms that serve as reservoirs for the development of pathogenic infections (*4*−*6*). In this context, many studies were performed to generate thin coatings that reduce bacterial adhesion onto solid surfaces. The developed methods are mainly based on two different strategies: (i) the immobilization of biocidal substances and (ii) the deposition of an antifouling coating that prevents proteins and subsequently cell adhesion on the surface. The techniques employed to immobilize antibacterial substances onto solid surfaces involve chemical grafting, surface impregnation, or physical entrapment (*7*−*11*). As for the nonadhesive coatings, they are mainly self-assembled monolayers (SAMs) (*12*−*24*) or polymer brushes based on poly(ethylene glycol) (PEG) or its derivatives (*15*−*17*). However, even though the antifouling properties of PEG-based layers have been widely reported in the literature, these layers do not completely prevent the adhesion of bacteria (*18*−*21*). Therefore, it is desirable to combine both approaches in effective antibacterial coatings: nonadhesive properties to reduce initial cell adhesion and the simultaneous presence of a biocidal substance to kill bacteria adhering onto the surface.

Recently, polymer brushes have attracted considerable attention as a way to engineer the surface properties of materials (*22*). By comparison with other surface modification methods (e.g., SAMs), they offer a higher mechanical and chemical robustness, which results in a higher long-term stability. A broad range of chemical functionalities can be obtained from an adequate choice of the monomers, as we demonstrated by testing the antibacterial properties of sulfonate brushes loaded with silver ions (*23*). Among techniques used to prepare polymer brushes, the "grafting from" approach based on surface-initiated atom transfer radical polymerization (ATRP) offers an experimentally straightforward route to dense brushes with a narrow polydispersity, a controlled architecture, and a well-defined thickness and composition (*24*). We have reported previously on the preparation of copolymer brushes based on 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) and oligo(ethylene glycol) methacrylate (OEGMA) (*25*−*27*). These PEG-based brushes are expected to be nontoxic, non-immunogenic, non-antigenic, and nonadhesive toward cells (*28, 29*). Here, similar copolymer brushes incorporating a hydroxyl-terminated oligo(ethylene glycol) methacrylate (HOEGMA) monomer were used as a platform to immobilize a natural antimicrobial peptide.

Among the substances used to inhibit the growth of bacteria, the natural defense peptides that are secreted by living organisms such as plants, microorganisms, and various mammalians, insects, and amphibians have recently emerged as a particularly promising class of biocidal drugs (*30*−*32*). Indeed, compared to conventional antibacterial substances such as antibiotics, quaternary ammonium compounds, or heavy metal derivatives, defense peptides have been shown to act at a much lower concentration, which considerably limits the potential problems related to toxicity (*33*−*35*). Moreover, these natural peptides have a broad spectrum of antimicrobial or even antifungal activity, and they rarely promote bacterial resistance. Recently, a few studies have reported on the immobilization of antibacterial peptides coupled to SAMs (*7*) or polyelectrolyte multilayers (*10, 11*) to prepare antibacterial thin films.

In this paper, we describe the oriented end-grafting of an antimicrobial peptide on poly(MEO_2MA-*co*-HOEGMA) brushes. Beside their nonadhesive behavior, these copolymer brushes offer reactive hydroxyl groups which allow controlled loading of the brushes with antimicrobial peptide grafted on the brushes and consequently the activity of the resulting coatings. Magainin

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I, a 23-residue defense peptide initially isolated from the skin of the African clawed frog *Xenopus laevis* (*36, 37*), was selected for its broad biocidal activity against Gram-positive and Gram-negative bacteria and its non-hemolytic properties at its effective antimicrobial concentration. Magainin-functionalized copolymer brushes of various compositions were tested against two different gram-positive bacteria.

EXPERIMENTAL SECTION

Materials. Di(ethylene glycol) methyl ether methacrylate (MEO_2MA) (95%), and hydroxyl-terminated oligo(ethylene glycol) ether methacrylate (HOEGMA) of molar mass ~360 g/mol were obtained from Aldrich and were used without purification. FITC-labeled streptavidin (streptavidin^{FITC}) and *N*-(*p*-maleimidophenyl)isocyanate (PMPI) were purchased from AbD Serotec and Appolo Scientific, respectively. Copper(I) chloride (99.995+%) ($Cu^{I}Cl$), copper(II) chloride (99.999+%) ($Cu^{II}Cl_2$), and 2,2′-dipyridyl (99+%) (bipy), tris(hydroxymethyl)aminomethane (tris) were from Aldrich. All solvents were distilled before use except methanol, which was analytical reagent grade. Milli-Q water (resistivity higher than 18.2 MΩ.cm) was obtained from a Millipore Simplicity 185 system. Silicon wafers (⟨100⟩ orientation) were obtained from Compact Technology Ltd.; they were cleaned for 10 min in an air plasma asher (100 W, Emitech K1050X) before use. Magainin I derivatives with an additional C-terminal cysteine residue and tagged with an N-terminal biotin moiety (Biotinyl-MAG-Cys) or untagged (MAG-Cys) were synthesized by the solid-phase methodology on Fmoc-Cys(Trt)-HMP resin by using a 433A Applied Biosystems peptide synthesizer and the standard Fmoc manufacturer's procedure. The synthetic peptides were purified by reversed-phase preparative HPLC on a Vydac 218TP1022 C_{18} column by using a linear gradient (10−60% over 60 min) of acetonitrile/trifluoroacetic acid (99.9:0.1; v/v) at a flow rate of 10 mL.min^{-1}. Analytical HPLC, performed on a Vydac 218TP54 C_{18} column, showed that the purity of the peptides was higher than 99.8%. The purified peptides were characterized by MALDI-ToF mass spectroscopy on a Voyager DEPRO in the reflecton mode with α-cyano-4-hydroxycinnamic acid as a matrix. LIVE/DEAD BacLight Bacterial Viability Kit L7007 containing SYTO 9 and propidium iodide dyes was purchased from Molecular Probes.

Preparation of the Antibacterial Brushes. *Initiator-Grafted Silicon Wafers.* The synthesis of the 2-bromo-2-methyl propionic acid 3-trichlorosilanylpropyl ester was adapted from a previously published procedures (*38*), and the formation of the initiator monolayer was performed as described previously (*39*). An ellipsometric thickness of 0.6−0.9 nm was found for the initiator monolayer (*25*).

Copolymer Brushes. The synthesis of the copolymer brushes by surface-initiated ATRP was adapted from a procedure recently published (*25*). Briefly, the monomers (95 mmol) were dissolved in a mixture of water (30 mL) and methanol (15 mL) (e.g., for a MEO_2MA/HOEGMA molar ratio of 67:33, 12 g of MEO_2MA (63.7 mmol) and 11.3 g of HOEGMA (31.3 mmol) were used) in a round-bottom flask sealed with a rubber septum. Bipy (5 mmol, 782 mg) and $Cu^{II}Cl_2$ (0.16 mmol, 21.5 mg) were added to this solution, which was stirred and degassed with a stream of nitrogen for 1 h. $Cu^{I}Cl$ (1.6 mmol, 158.5 mg) was then added quickly to the solution. The solution was stirred and degassed for 45 further min. Meanwhile, the initiator-grafted Si wafers (1 × 1 cm^2) were sealed into Schlenk tubes and were degassed (4 vacuum/nitrogen filling cycles). The polymerization solution was then syringed and quickly transferred to the Schlenk tubes. The Schlenk tubes containing the samples were kept at room temperature under an overpressure of nitrogen in the absence of stirring, for a given polymerization time that depends on the monomer ratio (from 3 h to 9 h). Then, the samples were removed, washed with water then methanol, dried with a stream of nitrogen, and stored under nitrogen before use. The polymerization times used for each monomer ratio were adapted to get copolymer brushes with an average thickness of about 110 nm (Table 1). Four different MEO_2MA/HOEGMA monomer ratios were tested to prepare the copolymer brushes: 100:0; 83:17, 67:33, and 33:67.

Table 1. Characteristics of the Poly(MOE_2MA-*co*-HOEGMA) Brushes

composition of the monomer mixture [MOE_2MA/HOEGMA]	reaction time (min)	brush thickness[a] (nm)
100:0	150	106
83:17	210	110
67:33	245	110
33:67	527	111

[a] Measured by ellipsometry.

Oriented Grafting of Magainin I Derivatives on Copolymer Brushes. Two milliliters of 6 mM PMPI solution prepared in distilled dry THF were transferred to a Schlenk tube containing the silicon wafer functionalized by the poly(MEO_2MA-*co*-HOEGMA) brush in a nitrogen-purged glovebox. After a reaction time of 6 h at room temperature, the silicon wafer was sonicated in distilled THF for 5 min, and the surface was thoroughly washed 3 times with distilled THF before finally being dried in a stream of nitrogen. Next, the PMPI-grafted brush was reacted with MAG-Cys or biotinyl-MAG-Cys derivative. For this, the silicon wafer was treated with a 280 μM solution of the peptide prepared in 100 mM tris buffer (pH 7.04). After a reaction time of 2 h 30 min at room temperature, the silicon wafer was sonicated 5 min in Tris buffer, thoroughly washed 3 times with Milli-Q water, and finally dried in a stream of nitrogen before use in bacterial assays.

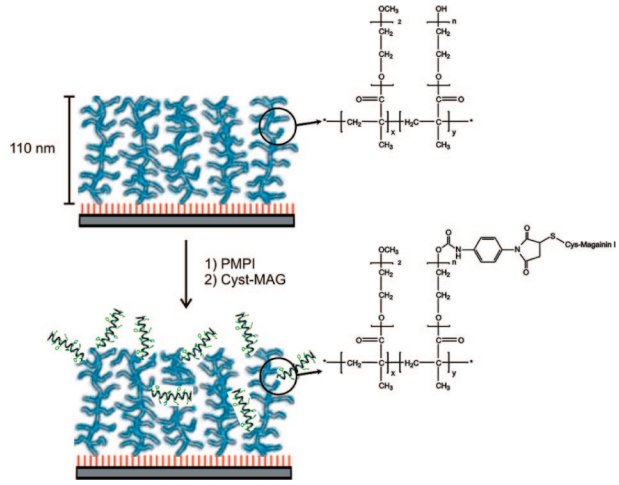
Analytical Techniques. *Ellipsometry.* Ellipsometric measurements were carried out with a spectroscopic ellipsometer (α-SE from J. A. Woollam Co., Inc.). The data were fit using a Cauchy model for the index of refraction of the organic layer, using tabulated values for the index of refraction of silicon. The 1.5-nm-thick layer of native silicon oxide was included in the organic layer, since they have close indices of refraction. Five measurements were taken and averaged for each sample. In most cases, the standard deviation of the thickness about its average value was lower than 3 nm.

Optical Microscopy. Optical and fluorescence images were taken with a Nikon Eclipse ME600L microscope equipped with a FITC filter set and an Epi-Fluorescence module, and connected to a DN100 image-capture system. Images were processed using *IGOR Pro* software (Wavemetrics, Inc.).

Confocal Laser Scanning Fluorescence Microscopy (CLSM). CLSM imaging was carried out with a Leica SP2 upright confocal laser scanning microscope (DM RXA2) equipped with an Acousto-Optical Beam Splitter (AOBS) system and using a 63× oil immersion objective with numerical aperture 1.32 (Leica Microsystems, Rueil-Malmaison, France). SYTO 9 was excited at 476 nm and observed from 485 to 540 nm, and propidium iodide was excited at 514 nm and observed from 580 to 670 nm. The laser power, the gain, and the offset for each photomultiplier have been adjusted to optimize the bacteria detection.

Images of the CLSM observations (1024 × 1024 pixels) have been acquired through the sequential mode to avoid fluorescence emission spectrum overlap, and the signal/noise ratio has been increased through line (×2) and frame (×4) averaging. Overlay images have been built with post-acquisition Leica Confocal software (LCS).

Scheme 1. Oriented Grafting of MAG-Cys Derivative on Poly(MOE$_2$MA-*co*-HOEGMA) Brushes via a PMPI Heterolinker



Fourier Transform Infrared Spectroscopy (FTIR). FTIR spectra were acquired between 1000 and 4000 cm^{-1} with a BioRad FTS-6000 spectrometer. The measurement were performed in transmission through the silicon wafers. The sample compartment was thoroughly purged with dry nitrogen before measurement.

X-ray Photoelectron Spectroscopy (XPS). XPS measurements were performed using a Kratos Axis Ultra spectrometer (Kratos Analytical) with a monochromatic Al Kα X-ray source ($h\nu$ 1486.6 eV). The pass energy was set at 40 eV for the detailed scans. In these conditions, the energy resolution gives a full width at half-maximum of the Ag $3d_{5/2}$ peak of about 0.1 eV. All binding energies were referenced to the *C*-(C,H) component of the C 1s peak fixed at 284.8 eV. The base pressure in the spectrometer was in the low 10^{-8} Torr range. Quantitative information was obtained by the photoemission peak areas of each element normalized according to acquisition parameters and sensitivity factors provided by the manufacturer. Peak decomposition was achieved with the Casa *XPS* software (Casa Software Ltd., UK), after subtraction of a linear baseline.

Bacterial Inhibition Assays. Magainin-functionalized brushes were tested against two Gram-positive bacteria: *Listeria ivanovii* (strain kindly provided by the UMR CNRS 6008 of University of Poitiers, France) and *Bacillus cereus* (strain kindly provided by the UMR CNRS 6143 of University of Rouen, France). Both bacteria strains were precultured in Mueller-Hinton broth for 18 h, then harvested by centrifugation (2000 × *g* for 15 min) and resuspended in distilled water. A functionalized silicon wafer was immersed in 10 mL of diluted bacteria suspension freshly prepared in distilled water (10^7 colony forming units (CFU).mL^{-1}). After 3 h incubation at room temperature, the silicon wafer was taken out and briefly rinsed with distilled water. To test the viability of bacteria adhering on the functionalized copolymer brush, the cells were immediately stained with the LIVE/DEAD BacLight Bacterial Viability Kit. For this, the wet silicon wafer was immersed in 2 mL of diluted viability kit solution. After an incubation time of 30 min, the silicon wafer was briefly rinsed with distilled water, and the surface was visualized by microscopy. The viability of the cells suspended in the distilled water was also controlled by counting the number of bacteria colonies grown on a seeded Mueller-Hinton agar medium (Difco). This control experiment confirmed that the viability of the bacteria was absolutely not affected after an incubation time of 3 h in water.

Culturability of sessile *L. ivanovvi* cells was also investigated. 1 × 1 cm^2 functionalized silicon wafers were deposited on 15 g/L κ-carrageenan plates (on which no bacterial growth occurs) and covered with a bacterial suspension (10^7 CFU.mL^{-1}). After incubation for 3 h at room temperature, wafers were picked up and rinsed with sterile distilled water. Adherent cells were recovered by sonication (two steps of 1 min at 50 W) of the material in 5 mL of sterile distilled water (Deltasonic bath, Meaux, France). The resulting cell population was enumerated by plating out decimal dilutions of the suspension on Plate Count Agar. After incubation for 24 h at 37 °C, the number of bacteria was expressed as CFU.cm^{-2} material.

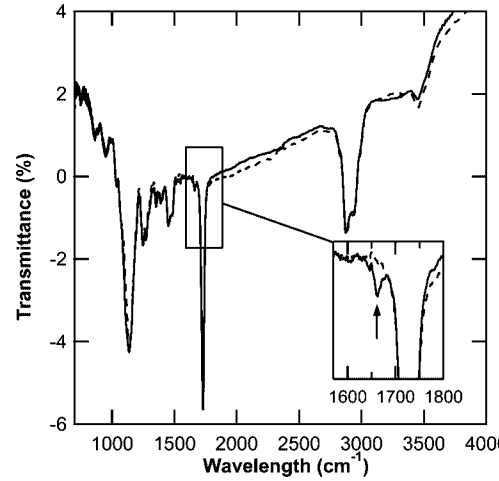


Figure 1. FTIR spectra of nonfunctionalized (dashed line) and MAG-Cys-functionalized (continuous line) poly(MOE$_2$MA-*co*-HOEGMA) [33:67] brushes. The arrow indicates the amide I absorption band.

RESULTS AND DISCUSSION

To prepare antibacterial coatings combining both biocidal and nonadhesive properties, we explored the tethering of a natural defense peptide, magainin I, on poly(MEO$_2$MA-*co*-HOEGMA) brush platforms. Because magainin I exerts its toxicity against bacteria by permeabilizing the cell membranes through pore forming or structural defects in membranes (*40, 41*), it is important to maintain its mobility and its accessibility after attachment on the copolymer brushes (*7*). To maintain the solution properties of magainin I upon attachment to the brushes, the peptide derivatives were tethered on the hydroxyl extremities of the relatively long water-soluble and flexible oligo(ethylene glycol) side chains of the poly(MOE$_2$MA-*co*-HOEGMA) brushes (Scheme 1). Furthermore, to orient the peptide on the surface and allow it to easily access bacterial membranes, a cysteine residue was attached to the C-terminal side of magainin I. Because magainin I does not contain cysteine residue, the magainin I derivative MAG-Cys could be exclusively attached to the brushes by its C-terminal part, via a PMPI heterolinker (Scheme 1): the isocyanate moiety of the linker reacts with the hydroxyl group of the copolymer brushes; the maleimide reacts with the thiol function of the MAG-Cys or Biotinyl-MAG-Cys derivative (*42, 43*). An interesting feature of the poly(MOE$_2$MA-*co*-HOEGMA) brushes is the possibility to vary their functionality in hydroxyl-reactive groups and subsequently the amount of immobilized magainin I, simply by changing the composition of the monomer mixture used. Various copolymer brushes of increasing content in reactive HOEGMA were thus prepared and subsequently functionalized by magainin I derivatives (Table 1). The reaction times used for the surface-initiated ATRP were adjusted to get ∼110-nm-thick brushes. The grafting of the MAG-Cys peptide on the copolymer brushes was confirmed by FTIR, which showed the occurrence of a new amide I absorption band centered at 1661 cm^{-1} after grafting (Figure 1). However, the poor signal/noise ratio obtained from these measurements does not allow us to quantify the real amount of peptide grafted

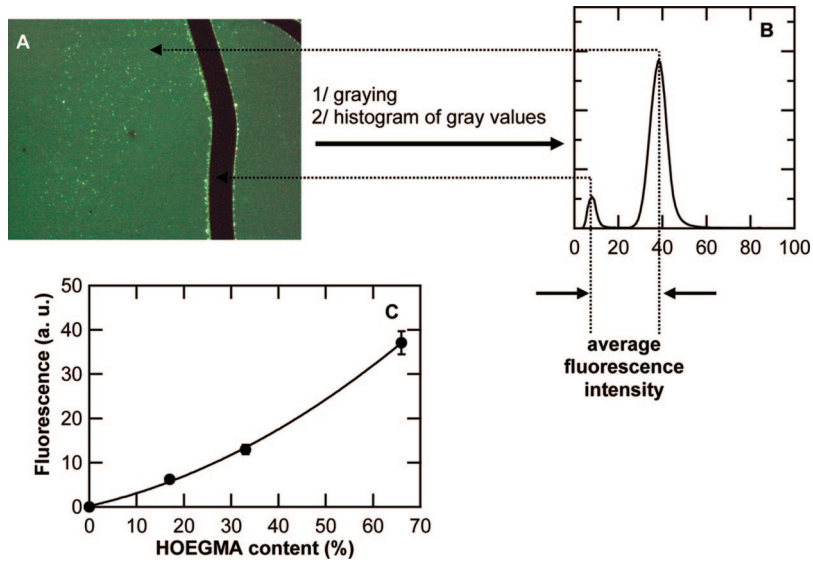


Figure 2. Image analysis of Biotinyl-MAG-Cys-grafted poly(MEO_2MA-*co*-HOEGMA) brushes incubated in a streptavidin^{FITC} solution: (A) Fluorescence image of a Biotinyl-MAG-Cys-grafted poly(MEO_2MA-*co*-HOEGMA) [33:67] brush; a deliberate scratch was made on the sample surface to determine the fluorescence background; (B) fluorescence intensity histogram computed from image (A) after graying; (C) fluorescence intensity of Biotinyl-MAG-Cys-grafted poly(MEO_2MA-*co*-HOEGMA) brushes vs their composition.

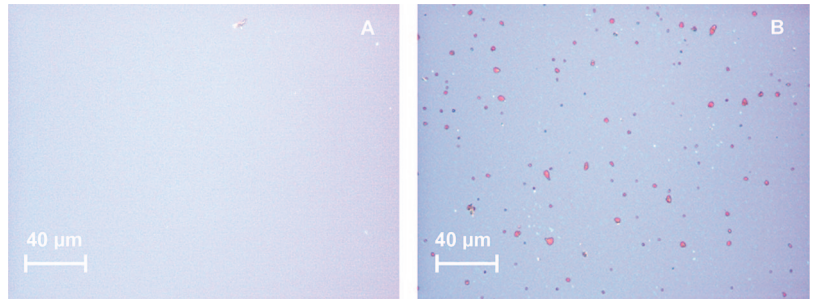


Figure 3. Optical micrographs of the brush surface after 3 h incubation in *L. ivanovii* suspension: (A) nonfunctionalized and (B) MAG-Cys-functionalized poly(MEO_2MA-*co*-HOEGMA) [33:67] brushes.

on the copolymer brushes. The percentage of monomer unit grafted by magainin was quantitatively estimated by XPS to be about 5% for a poly(MOE_2MA-*co*-HOEGMA) brush [67:33]. This percentage is significantly lower than the content of the brush in hydroxyl grafting moieties, which strongly supports the notion that the grafting reaction essentially occurs on the brush surface. To estimate qualitatively the influence of the composition of the copolymer brush on the amount of magainin I grafted on the brush surface, the brushes were functionalized with a biotin-tagged derivative (Biotinyl-MAG-Cys) and were subsequently incubated with streptavidin^{FITC}. Then, the surface-immobilized streptavidin^{FITC} was detected by fluorescence microscopy. For all Biotinyl-MAG-Cys-functionalized brushes tested, the surfaces were uniformly fluorescent on a micrometer scale, which indicates the uniform immobilization of the biotin-tagged magainin I molecules on the surface (Figure 2). In contrast, the absence of fluorescence of brushes not functionalized by magainin I (result not shown) testified to their inertness toward protein adsorption. Therefore, the fluorescence detected on functional brushes arises from the streptavidin binding to the biotin-tagged brushes, not from unspecific adsorption. The amount of grafted peptide was indirectly estimated as a function of the composition of the copolymer brush (e.g., HOEGMA content) by extracting the average fluorescence intensity by image analysis (Figure 2). For this, a deliberate scratch was made on the sample surface to determine the fluorescence signal-to-background (Figure 2). After graying the recorded image and computing the histogram of gray values, the fluorescence intensity was extracted as shown in Figure 2. The results displayed in Figure 2 show that the amount of streptavidin^{FITC} bound to the Biotinyl-MAG-Cys-functionalized brushes increases with the amount of HOEGMA in copolymer brush. As a consequence, the higher the content in hydroxyl reactive group in the brush, the higher the amount of magainin derivative grafted. This result confirms that the amount of tethered magainin I can be tuned by simply varying the composition of the copolymer brush used as a platform. Such a design is of a particular interest to optimize the biocidal properties of the surface.

The antibacterial properties of the MAG-Cys-modified brushes were tested toward two strains of Gram-positive bacteria: *L. ivanovii*, which causes serious food intoxications (*44*), and *B. cereus*, which is also involved in food-borne diseases and exhibits a high ability to adhere and to form biofilms on stainless steel (*45*) and glass (*46*). Experimentally, the functionalized silicon substrates were incubated for 3 h in bacteria suspension and immediately observed by optical microscopy. For the sake of comparison, a control adhesion test with unmodified copolymer brushes was also performed. The inspection of recorded micrographs (Figure 3) revealed the absence of bacteria on the unmodified poly(MOE_2MA-*co*-HOEGMA) [33:67] brush for both strains tested (results shown only for *L. ivanii*), which testifies to the inertness of this brush platform. A similar result was obtained for unmodified copolymer brushes of varying composition. In contrast, some bacteria were observed on magainin-functionalized brushes, but the cell coverage did not exceed 1% of the surface, independent of the copolymer brush composition. This result indicates that the bacterial attachment

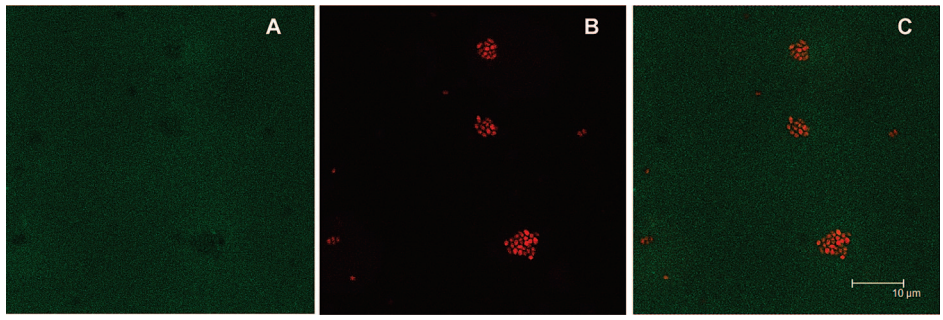


Figure 4. CLSM images of MAG-Cys-functionalized poly(MEO_2MA-*co*-HOEGMA) [33:67] brush incubated in the presence of *L. ivanovii* and subsequently stained with the LIVE/DEAD viability kit: (A) green channel image corresponding to alive stained bacteria; (B) red channel image corresponding to dead bacteria; (C) overlay image built from (A) and (B).

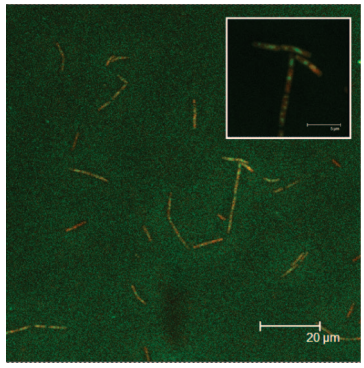


Figure 5. CLSM overlay image of MAG-Cys-functionalized poly(MEO_2MA-*co*-HOEGMA) [33:67] brush incubated in presence of *B. cereus* and subsequently stained with the LIVE/DEAD viability kit. The overlay image was built from both red channel and green channel images. Inset: An enlarged image of stained bacterial cells.

onto the brushes is exclusively due to the presence of the magainin I peptide, which interacts with cell membranes. To check the viability of the bacteria observed on the magainin-functionalized brushes, the adhering cells were stained with the fluorescent LIVE/DEAD viability kit before CLSM observation (*47*). The inspection of the green and red channels of the CLSM images obtained on magainin-functionalized brushes exposed to *L. ivanovii* (Figure 4) revealed only the presence of red-stained cells corresponding to dead bacteria. This observation was made even for the sample of lower grafting ratio (results not shown). Similar assays were performed on *B. cereus*. CLSM observations showed that every single *B. cereus* cell adhering to the surface of the functionalized brushes is partially red-stained (Figure 5), which testifies to the presence of damages to its membrane. The fact that *B. cereus* cells were not seen to be completely red-stained is most probably due to the staining process, which depends on the conditions and on the bacteria used, as was previously noted (*48, 49*). Bacterial cell counts performed with *L. ivanovii* after 3 h of contact, confirmed the antimicrobial properties of the magainin-grafted brushes. Indeed, a colonization of 700−800 $CFU.cm^{-2}$ was measured, independent of the composition of the copolymer brush. Sessile bacteria represents less than 0.0001% of the planktonic population, a ratio close to those reported by Kebir et al. (*50*) with *Pseudomonas aeruginosa* on antibacterial cross ionic copolyurethanes, and much lower than those generally obtained in same experimental conditions with raw materials (*50, 51*). Thus, the assays performed on both tested bacterial species demonstrate the high efficiency of the magainin-functionalized copolymer brushes to kill all the bacterial cells adhering on their surface. The biocidal activity was detected even for the lower grafting ratio in magainin I peptide tested.

One might wonder about how the grafted peptide molecules exert their antimicrobial effect. The detailed mode of antibacterial action of magainin is still unclear. However, it seems obvious that the membrane barrier properties are reduced by the presence of magainin molecules in the membrane, even though experimental data do not support the formation by magainin of well-defined membrane channels (*52*). As a consequence, the accumulation of magainin at the surface of the brushes combined with the flexibility of the HOEGMA side chains is probably sufficient to allow an antimicrobial effect, which is compatible with current models of magainin action, such as transmembrane helical bundles, toroidal model, carpet model, and the detergent-like peptide model (*52*). It has to be noted that biocidal activity of an antimicrobial peptide immobilized onto a solid surface via an oriented chemical grafting was also previously reported (*7*). Therefore, the magainin-functionalized brushes provide an efficient protection against bacterial spreading by killing cells in contact with them. The presence of the antimicrobial peptide combined with the intrinsic antifouling properties of the brushes should result in a strong delay of Biofilm formation, which is of interest for many applications in the medicine and food industries.

CONCLUSION

Bacterial biofilms cause infections as well as bacterial and chemical contaminations in many domains, from food and water industries to the biomedical field. On biomaterials, in particular, they often lead to removal of infected implants, because biofilms protect bacteria against antibacterial treatments and store bacteria for further infections. Among the possible methods of action against Biofilm formation, prevention by the control of the first stage of its formation (primary colonization of bacterial adhesion) is considered to be the most efficient strategy. Here, we described an original approach combining the antiadhesive property of poly(MEO2MA-*co*-HOEGMA) brushes and the antibacterial activity of an immobilized natural peptide to prepare efficient antibacterial coatings. Surface-initiated ATRP was used to synthesize nonadhesive poly(MEO2MA-*co*-HOEGMA) brushes that were subsequently used as platforms to covalently immobilize a natural antibacterial peptide. We demonstrated that the peptide can be tethered in various densities by changing the composition of the copolymer brushes, which allows variation of the biocidal activity of the coatings. The highly biocidal activity of the peptide-functionalized brushes was in evidence even for low levels of grafted peptide. The strategy developed here should be easily adaptable to coat various materials or items used in the medicine and food industries. Moreover, this approach could be generalized to other natural peptides to prepare thin coatings with a broad spectrum of activity against bacteria, fungi, and protozoa.

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