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Structure−Activity Relationship for Quaternary Ammonium Compounds Hybridized with Poly(methyl methacrylate)

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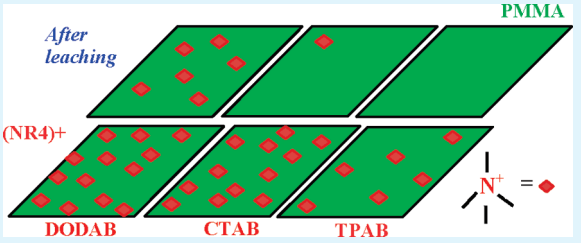
Supporting Information

ABSTRACT: Hybrid films from poly (methylmethacrylate) (PMMA) and dioctadecyldimethylammonium bromide (DODAB), cetyltrimethylammonium bromide (CTAB), or tetrapropylammonium bromide (TPAB) were characterized by determination of wettability, ellipsometry, atomic force microscopy, active compounds diffusion to water, X-ray photoelectron spectroscopy (XPS) with determination of atomic composition on the films surface, and biocidal activity against *Pseudomonas aeruginosa or Staphylococcus aureus*. QAC mobility in the films increased from DODAB to CTAB to TPAB. Diffusion and optimal hydrophobic−hydrophilic balance imparted the highest bioactivity to CTAB. DODAB sustained immobilization at the film surface killed bacteria upon contact. TPAB ability to diffuse was useless because of its unfavorable hydrophobic−hydrophilic balance for bioactivity.



KEYWORDS: hybrid antimicrobial surfaces, dioctadecyldimethylammonium bromide, cetyltrimethylammonium bromide, tetrapropylammonium bromide, poly (methyl methacrylate), XPS

INTRODUCTION

Polymeric devices and biomaterials play an important role in the transmission of infectious diseases because of formation of biofilms on their surfaces.[1] Bacteria from the biofilm may often initiate infection in adjacent regions.[2] These surface-associated contaminants organized as biofilms exhibit increased resistance to detergents, disinfectants, or antibiotics and provide microbial protection against immunological defenses.[1,2] Much work has been devoted to hybrid antimicrobial materials with improved properties. Three types of antimicrobial surfaces have been developed: (1) those killing microorganisms upon contact;[3,4] (2) those preventing microbial adhesion onto the surface;[5] (3) those leaching antibacterial agents.[6] Antimicrobial materials have been designed in form of particles,[7] coatings or films,[4] or permanent nonleaching antibacterial surfaces able to prevent contamination.[8−10]

A variety of cationic antimicrobials have been effective such as peptides[11] and long-chained surfactants and lipids.[12−14] Molecules with a net positive charge killed microorganisms both in solution[11] or upon adsorption[4,7] or grafting to flat surfaces or particles.[3,9,10]

Antimicrobial coatings have been prepared by different strategies such as the layer-by-layer deposition,[7] grafting or covalent binding of antimicrobial moieties to surfaces[3,9,10] or spin-coating technique to impregnate thin polymeric films with dioctadecyldimethylammonium bromide (DODAB).[4] The last approach was based on solubility of polymer and antimicrobial agent in a common solvent, e.g., chloroform and spin-coating of the solution on a supporting surface yielding thin, smooth and homogeneous films. Thereby the antimicrobial DODAB properties[13] were transferred to the hybrid polymer/DODAB coating and *Escherichia coli* was effectively killed upon contact.[4]

Poly (methyl methacrylate) (PMMA) has been widely used in medical and dentistry devices,[15,16] where formation of biofilms was frequently reported.[17] In this work, spin-coated hybrid films prepared from PMMA and one of three different QACs, DODAB, cetyltrimethylammonium bromide (CTAB) or tetrapropylammonium bromide (TPAB) (see Scheme 1 for chemical

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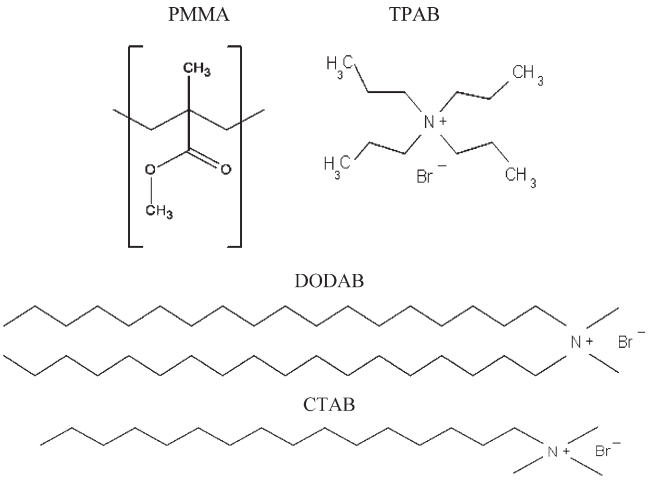
Scheme 1. Chemical Structures of Polymer and QACs in the Hybrid Films



structures) were characterized regarding atomic composition, film thickness, mean surface roughness (rms), wettability, QAC diffusion from film to water and antimicrobial activity. Methods were XPS, ellipsometry, atomic force microscopy (AFM), sessile drop contact angle, Du Noüy ring for surface tension (γ) at the air−water interface, colony forming unities (CFU) counting, and evaluation of inhibition zone. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were selected for antimicrobial activity testing due to their well-known role in biofilm formation. This work reveals for the first time the relationship between chemical structure of the QAC in the film and bioactivity against bacteria. CTAB diffusion from films and optimal hydrophobic hydrophilic balance imparted the highest antimicrobial activity in comparison to DODAB or TPAB. DODAB large affinity for the polymer even after extensive leaching killed bacteria upon contact. TPAB unfavorable hydrophobic−hydrophilic balance with poor affinity for interfaces resulted in absence of any antimicrobial activity.

EXPERIMENTAL SECTION

Materials. Dioctadecyldimethylamonium bromide (DODAB) 99.99% pure, cetyltrimethylammonium bromide (CTAB) and poly (methylmethacrylate) ~120 000 of molecular weight (PMMA) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Tetrapropylammonium bromide (TPAB) was obtained from Merck (Schuchardt, Germany). Chloroform was analytical grade and used without further purification. The glass coverslips used as substrates for deposition of the films were 20 × 20 mm squares with thickness of 0.13 to 0.16 mm. Si wafers (1 cm × 1 cm) with native SiO_2 layer were purchased from University Wafers (Boston, USA). Prior to use they were rinsed in oxidative medium, as described elsewhere.[4]

Film Preparation. Chloroform was previoulsy described as a good solvent for both PMMA and DODAB (4). Chloroformic solutions of PMMA and quaternary ammonium compounds (QACs) were prepared at 10 mg/mL PMMA and QAC concentrations ranged from 0.03 to 3.8 mM, yielding 0.01875−2.4 mg/mL DODAB, 0.01092−1.38 mg/mL CTAB, and 0.008−1.011 mg/mL TPAB. Glass coverslips and Si/SiO_2 wafers were spin-coated with chloroform before spin-coating 0.1 mL of the PMMA/QAC chloroformic solution on support. The final mass of material on the support was calculated as deposited material on the support per unit area (Max mass, in $\mu g/cm^2$ or in $\mu mols/cm^2$) assuming no losses of solution by spin-coating. All coatings were obtained by means of a Headway PWM32-PS-R790 spinner (Garland, USA), operating at 3000 rpm during 30 s, 24 ± 1 °C, and 50 ± 5% of relative humidity.

Film Characterization. Hybrid PMMA/DODAB films on silicon wafers were previously characterized by ellipsometry, wettability, optical and atomic force microscopy, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and activity against *Escherichia coli*.[4] In this work, PMMA/QAC hybrid films on glass coverslips were characterized regarding wettability by determining advancing and receding contact angles of a sessile water droplet placed on their surface. The measurements of contact angles were performed at 24 ± 1 °C in a home-built apparatus, as previously described.[4] Droplets of 8 μL of ultrapure water with a resistivity of 18 M Ω cm at 25 °C (doubly distilled and deionized water obtained from a Milli-Q apparatus) were deposited onto the dry film for determining the advancing contact angles (θ_A).

The diffusion of QACs from the films into the water solution was evaluated from surface tension at the air−water interface (γ) as a function of time (0.5−48 h). Spin-coated films on glass coverslips were immersed in 10 mL of ultrapure water inside a Teflon recipient at 24 ± 1 °C, and thereafter measurements were obtained by the Du Noüy ring method using a Byk-Labotron Dynometer (Germany).

Ellipsometric measurements[18] were performed ex situ (in air), at 24 ± 1 °C, using a vertical computer-controlled DRE-EL02 Ellipsometer (Ratzeburg, Germany). The angle of incidence was set to 70.0° and the wavelength of the He−Ne laser was 632.8 nm with an incidence area of 3 mm^2. Ellipsometric angles Δ and Ψ were measured and recorded at intervals of 4 s. For the interpretation of the ellipsometric angles Δ and Ψ a multilayer model was used, composed by the substrate, the unknown layer and the surrounding medium. Thickness (d) and refractive index (n) of the unknown layer could be calculated from the ellipsometric angles Δ and Ψ, using the fundamental ellipsometric eq 1 and iterative calculations with Jones matrices[18]

$$e^{i\Delta}\tan\Psi = R_p/R_s = f(n, d, \lambda, \phi) \quad (1)$$

where R_p and R_s are the overall reflection coefficients for the parallel and perpendicular polarization. They are a function of incidence angle (Φ), incident wavelength (λ), refractive index (n), and thickness of each layer of the model (d).

In this work, a multilayer model for silicon, silicon dioxide, adsorbed hybrid film layers and surrounding medium (air) was used. Initially, the refractive index n for Si layer was assumed as 3.858−0.018 i and its thickness, as an infinite one.[19] For the surrounding medium the refractive index was considered as 1.00. Because the native SiO_2 layer is very thin, its n was set as 1.462,[19] and the thickness of SiO_2 layer (d) was determined for each film. For PMMA film and for hybrid films of PMMA/DODAB, PMMA/CTAB, or PMMA/TPAB, the values of n and d were determined by iterative calculations.

Atomic force microscopy (AFM) measurements were performed in a PICO SPM-LE (Molecular imaging) microscope in the intermittent contact mode (AAC mode) in air, at room temperature, using silicon cantilevers with resonance frequency close to 300 kHz. Scan areas varying from (10 × 10) μm^2 to (2 × 2) μm^2 were obtained with resolution of 512 × 512 pixels. Image processing and root-mean-square (rms) roughness for the films were obtained from the PicoScan 5.3.2.software.

XPS measurements were performed using a K-Alpha XPS spectrometer (ThermoFisher Scientific, East Grinstead, U.K.). Data acquisition and processing using the Thermo Avantage software is described elsewhere.[20] All films were analyzed using a microfocused, monochromated Al Kα X-ray source (30−400 μm spot size). The K-Alpha charge compensation system was employed during analysis, using electrons of 8 eV energy and low-energy argon ions to prevent any localized charge build-up. The spectra were fitted with one or more Voigt profiles (BE uncertainty: +0.2 eV). The analyzer transmission function, Scofield

sensitivity factors,[21] and effective attenuation lengths (EALs) for photoelectrons were applied for quantification. EALs were calculated using the standard TPP-2 M formalism.[22] All spectra were referenced to the C1s peak of hydrocarbon at 285.0 eV binding energy controlled by means of the well-known photoelectron peaks of metallic Cu, Ag, and Au, respectively.

Organisms and Culture Conditions. Strains of *Pseudomonas aeruginosa* ATCC (American Type Culture Collection) 27853 and *Staphylococcus aureus* ATCC 25923 were separately reactivated for 2−5 h at 37 °C in 3 mL of Tryptic Soy Broth TSB (Merck KGaA, Darmstadt, Germany). Thereafter, bacteria were spread on plates of Mueller-Hinton Agar MHA (Hi-Media Laboratories Pvt, India) and incubated at 37 °C for 24 h. Two or three isolated colonies of each species were taken from the plates and shaken in 10 mL of TSB at 160 rpm and 37 °C for 2−3 h. Thereafter, each culture was pelleted and separated from its nutritive medium by centrifugation (8000 rpm, 15 min). The supernatant was withdrawn and replaced by an isotonic solution of 0.264 M D-glucose for resuspending the bacteria pellet. This centrifugation/resuspension procedure was repeated twice before using the bacteria for evaluating the antimicrobial activity of PMMA/QAC films. Turbidity of the bacteria suspension at 625 nm was adjusted to 0.5 of the McFarland scale, yielding a final cell concentration of $\sim(2-5) \times 10^7$ CFU/mL. The 0.264 M D-glucose solution is equivalent to a 0.15 M NaCl solution regarding osmolarity and thus said isotonic, and was used to preserve isotonicity between the internal and the external bacterial cell compartments.

Determination of Antimicrobial Activity. Antimicrobial activity of the hybrid films was evaluated by two different procedures: CFU counting and inhibition zone determination. In order to sterilize the glass coverslips coated with the hybrid films, substrates covered by films were exposed to UV light for 30 min on each side in a laminar flow cabinet just before determining antimicrobial activity. Activity of PMMA/QAC films was evaluated from plating and colony forming unit (CFU) counting after 1 h of interaction between bacteria and film. The protocol for testing antimicrobial activity was previously described.[23] The bacterial suspension was diluted 100-fold in 0.264 M D-glucose solution to achieve 1×10^5 to 1×10^6 CFU/mL. A 60 μL inoculum of either *P. aeruginosa* or *S. aureus* was placed on the film surface, covered with a second bare glass coverslip and incubated at room temperature, in a humid chamber, for 1 h. Thereafter, the film was immersed in 10 mL of a 0.264 M D-glucose solution inside a centrifuge tube. The tube was vortexed for 1 min to remove the bacteria from the film surface. The resulting suspension was diluted (0-fold and 10-fold) before 100 μL was plated in triplicate on MHA. After incubating for 24 h at 37 °C, CFU counting on agar was performed.

The inhibition zone for the hybrid films was determined against the same bacteria quoted above. Petri dishes containing MHA were sown with bacterial suspensions using a swab. The films gently contacted the seeded agar for 24 h, at 37 °C. Inhibition zone was detected visually by no growth of the model bacteria used. Alternatively, inhibition zone (no bacterial growth) around the film was measured, at least 10 times, from the rim of the glass coverslip to the zone of bacterial growth.[24] Appropriate controls with PMMA films or bare glass coverslips were performed. All experiments were done at least in duplicate.

RESULTS

Advancing contact angles (θ_A) decreased as a function of [QAC] for DODAB or CTAB (Figure 1). For TPAB, θ_A dependence on [TPAB] in the hybrid films was weak (Figure 1A, B). Thus, wettability increased as a function of QAC concentration in the

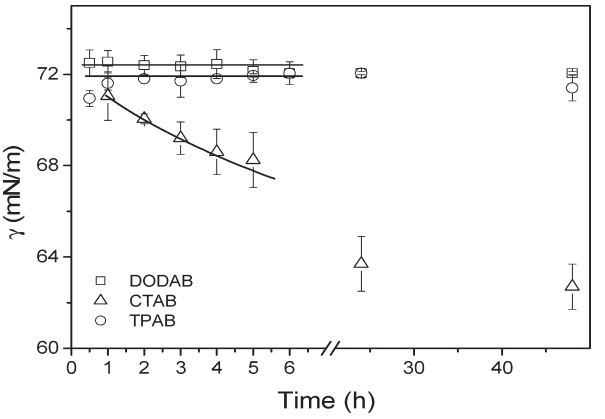


Figure 2. Surface tension (γ) at the air−water interface as a function of time elapsed after immersing PMMA/QAC film in 10 mL of pure water. QAC was DODAB (□), CTAB (Δ), or TPAB (○). Films were casted on glass coverslips from a chloroformic PMMA/QAC solution (10 mg/mL PMMA and 3.8 mM QAC).

Table 1. Physical Properties of Hybrid Films on Silicon Wafers for Films Prepared from 10 mg/mL PMMA and 3.8 mM QAC Chloroformic Solutions

film composition	n^a	d^b (nm)	rms^c (nm)	$\theta_A{}^d$ (deg)
PMMA	1.44 ± 0.01	82 ± 4	0.4	64 ± 3
PMMA/TPAB	1.48 ± 0.01	89 ± 5	0.4	60 ± 3
PMMA/DODAB	1.52 ± 0.01	102 ± 5	1.2	47 ± 3
PMMA/CTAB	1.48 ± 0.01	93 ± 5	1.7	42 ± 1

[a] Refractive index (n). [b] Thickness (d). [c] Mean surface roughness (rms). [d] Advancing (θ_a) contact angle.

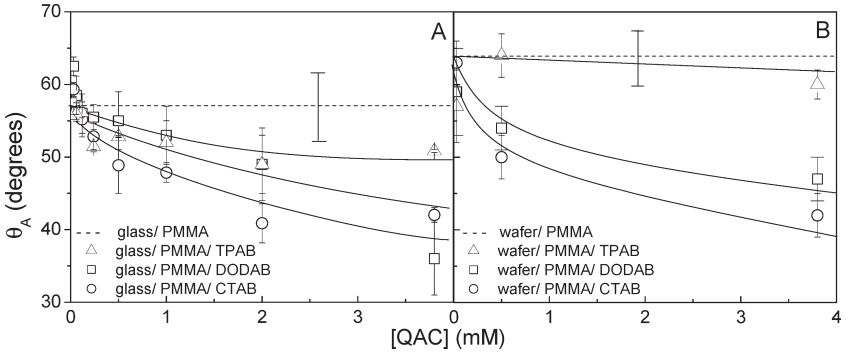


Figure 1. Contact angle (θ_A) for PMMA/QAC films on glass coverslips (A) or silicon wafers (B) as a function of [QAC]. QAC was DODAB (□), CTAB (Δ), or TPAB (○). The dashed line represented contact angles for pure PMMA films.

films and this increase was more pronounced for DODAB and CTAB.

To evaluate QAC diffusion from the film to the aqueous phase, kinetics of surface tension at the air−water interface (γ) at the largest QAC concentration employed was determined (Figure 2). For PMMA/DODAB or PMMA/TPAB films, surface tension was constant with time and equal to the surface tension of water (72 mN/m). For PMMA/CTAB films, surface tension decreased with time after immersing the film in water (Figure 2).

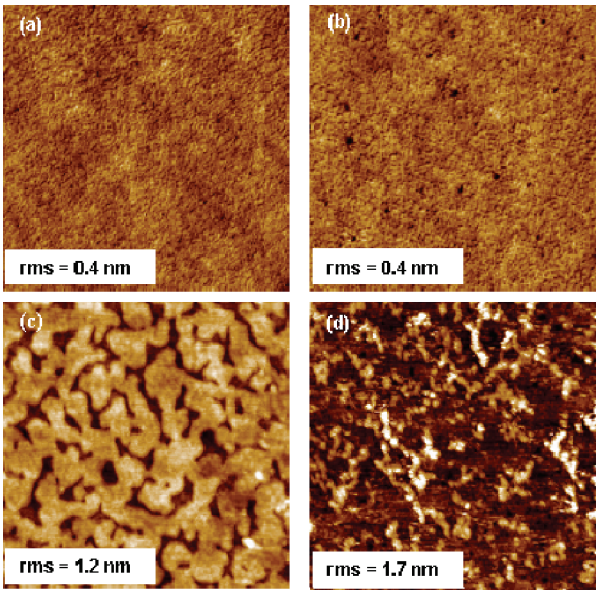


Figure 3. AFM topographic images (2 μm × 2 μm) obtained for spin-coated films onto Si/SiO_2 wafers. (a) PMMA, Z = 5 nm; (b) PMMA/TPAB, Z = 5 nm; (c) PMMA/DODAB, Z = 10 nm; (d) PMMA/CTAB, Z = 10 nm. PMMA concentration in the chloroformic solution was 10 mg/mL and QAC concentration was 3.8 mM.

Physical properties of hybrid films casted on silicon wafers were in Table 1. Ellipsometry allowed determination of film thickness (d) and refractive index (n). Values of d varied with QAC nature. CTAB or DODAB in the film composition significantly increased film thickness from 82 to 93 or 102 nm, respectively (Table 1). Furthermore mean surface roughness (rms) also increased because of DODAB or CTAB (Table 1). In contrast, TPAB barely affected rms values for the hybrid films. Films with TPAB presented wettability slightly smaller than the ones measured for control PMMA films, whereas DODAB or CTAB significantly increased the wettability of hybrid films (Table 1).

From AFM images, PMMA or PMMA/TPAB were smooth (Figure 3 a, b) and PMMA/DODAB or PMMA/CTAB films were rough (Figure 3 c, d).

Cell viability as a function of QAC concentration in PMMA/QAC films showed their bactericidal activity against *P. aeruginosa* and *S. aureus* (Figure 4A, B). PMMA/DODAB and PMMA/CTAB films exhibited antimicrobial activity in contrast to PMMA/TPAB films that did not affect cell viability.The intrinsic activity of CTAB and TPAB in solution were also evaluated (Figure 4C, D), though a direct comparison with QAC effects from the films cannot be performed. TPAB did not affect cell viability for both bacteria.

DODAB or CTAB doses for killing 99 or 50% of bacteria after 1 h interaction were shown in Table 2. CTAB was required at smaller doses than DODAB. *S. aureus* was more sensitive than *P. aeruginosa* to CTAB or DODAB in the films. The QAC mass for 99 or 50% killing (1 h interaction) was also shown in Table 2. DODAB doses for killing bacteria were always higher than those for CTAB (Table 2).

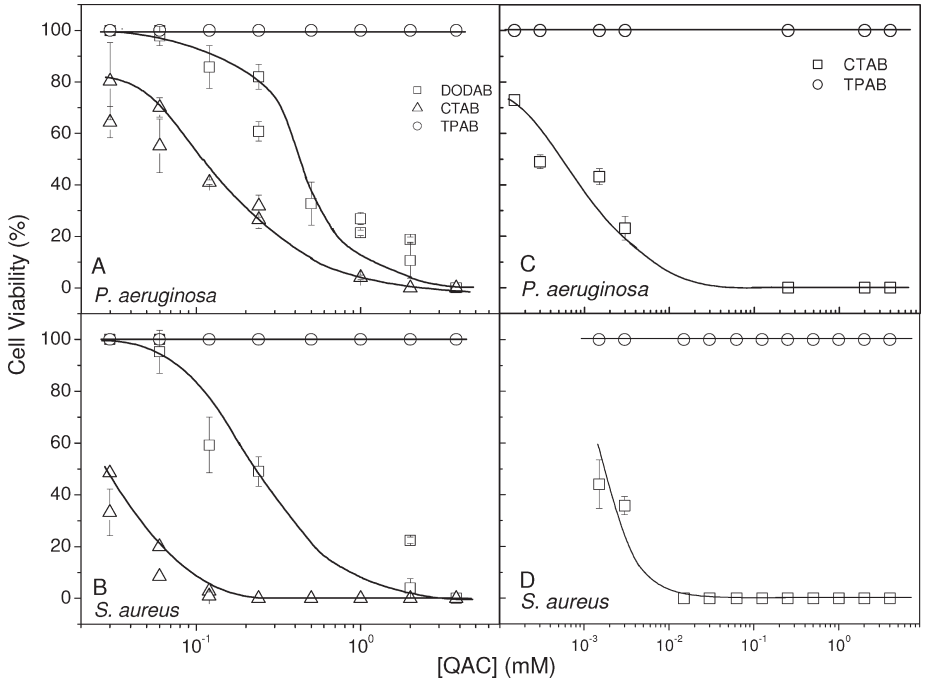


Figure 4. Cell viability (%) as a function of [QAC] in: PMMA/QAC chloroformic solutions used to produce the hybrid films by spin-coating (A, B) or QAC dispersions only (C, D). Bacteria and films or QAC dispersions interacted for 1 h at 1.5−3.0 × 10^7 CFU/mL of *P. aeruginosa* or *S. aureus*. Controls for pure PMMA films or bare coverslips yielded 100% of cell viability.

Table 2. QAC Concentration in the Chloroformic Solution Used for Spin-Coating Hybrid Films Yielding 99 or 50% Cell Death (1h interaction, 10^6 CFU/mL)[a]

		$[QAC]_{99/50}$		$(QAC\ mass/cm^2)_{99/50}$[b]	
hybrid film	bacteria	mg/mL	mM	$\mu g/cm^2$	$\mu mol/cm^2$
PMMA/DODAB	*P.aeruginosa*	2.2/0.2	3.5/0.3	55/5	87/8
DODAB		0.014/0.003	0.02/0.005		
PMMA/CTAB		0.5/0.04	1.4/0.1	13/1	35/3
CTAB		0.091/0.0002	0.25/0.0006		
PMMA/DODAB	*S.aureus*	2.0/0.1	3.2/0.2	50/3	80/4
DODAB		0.017/0.005	0.027/0.008		
PMMA/CTAB		0.05/0.01	0.14/0.03	1.2/0.3	4/1
CTAB		0.005/0.0007	0.015/0.002		

[a] Intrinsic activity of QACs alone is also shown. Data for DODAB intrinsic activity against both bacteria were taken from the literature.[25]. [b] Mmass of QAC per cm^2 was calculated from the volume and concentration of chloroformic solution spin-coated on the support.

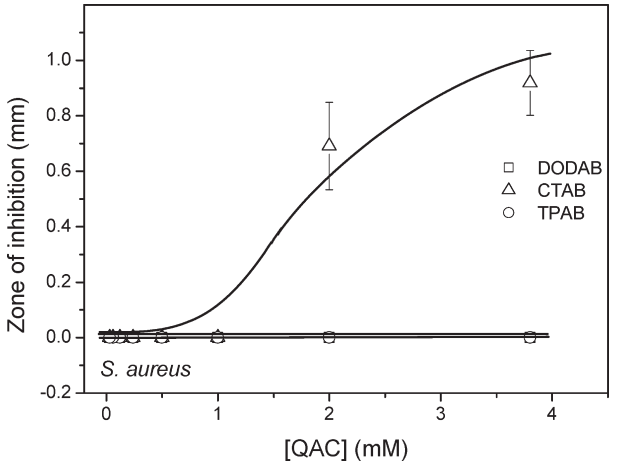


Figure 5. Inhibition zone of *S. aureus* as a function of [QAC] in the chloroformic solution used for spin-coating PMMA/QAC films.

QACs diffusion across the film and through the agar was determined from inhibition zone experiments (Figure 5). Against *S. aureus*, PMMA/DODAB or PMMA/TPAB films did not produce any inhibition zone in contrast with PMMA/CTAB over a range of QACs concentration. For *P. aeruginosa*, a similar experiment revealed that inhibition zones were absent for all films. This showed the CTAB ability to diffuse across the agar.

To quantify atomic composition at the film interface before and after exposure to water solution, we performed XPS analysis before and after film immersion in water (Table 3). One should notice the major effect of extensive leaching of QACs from the films. Quaternary nitrogen percentiles decreased to zero or very small relative values for TPAB and CTAB, respectively. For DODAB, about 30% of the initial value still remained at the film surface after extensive leaching, showing the large DODAB affinity for the PMMA polymer. After leaching, quaternary nitrogen of TPAB or CTAB were barely found at the film surface (Table 3). Before leaching, bold figures in Table 3 for Br should be compared with those for quaternary nitrogen also in bold. The percentiles of Br and quaternary nitrogen are practically the same showing the obvious association between the quaternary amonium polar head and its bromide counterion for films as prepared. Curiously, after leaching, though the quaternary ammonium belonging to DODAB still remained at the film surface, its counterion no longer appeared to be this halide since bromide concentration at the film surface turned out to be zero after leaching (Table 3). Possibly, OH^- from water replaced bromide. Other interesting aspect of atomic composition at the film surface was the large increase in C−H moieties for PMMA/DODAB films as prepared in comparison to PMMA films. This confirmed localization of DODAB hydrocarbon chains at the interface. The same trend was observed for PMMA/DODAB films after leaching.

Major peaks on XPS spectra were in very good agreement with the previous XPS literature for PMMA,[26,27] $(NR4)^+$,[28,29] CH, CO and COO,[30] C−N−H/O=C−N−H,[31−33] and Br.[34] All XPS spectra for the hybrid films as prepared and after leaching are available in the Supporting Information.

DISCUSSION AND CONCLUSIONS

The increase in wettability of PMMA/QAC films with QAC concentration was due to exposure of quaternary ammonium polar moieties to the film-water interface (Figure 1). This exposure increased from TPAB to CTAB to DODAB at a given QAC concentration (Table 3). Indeed, XPS data revealed that TPAB concentration at the film−air interface was about three times smaller than the one determined for CTAB and 4 times smaller than the one determined for DODAB (Table 3). The three QACs were able to leach to the water solution (Table 3), however, DODAB revealed its highest affinity for the film by remaining at significant concentrations on the film surface even after extensive leaching (Table 3).

CTAB was required at smaller doses than DODAB for killing the tested bacteria (Table 2), possibly due to its lower affinity for the films, higher diffusibility and appropriate hydrophobic−hydrophilic balance. It was reported in the literature that excessive length of alkyl substituents of QAC groups can result in a decrease in the activity, since intermolecular hydrophobic associations between DODAB molecules might be preventing interactions with bacteria.[35] The major DODAB advantage relative to CTAB was its higher affinity for the film interface allowing steady exposure of bioactive quaternary nitrogen to the bulk solution.

CTAB diffusion from the film to the agar shown in Figure 5 was also reported for multilayers of anionic poly(acrylic acid) and CTAB.[24] From Figure 5, Gram-positive were more sensitive than Gram-negative bacteria regarding killing upon contact with antimicrobial cationic surfaces, again in accordance with the literature.[24] Cationic amphiphiles killed more easily the Gram-positive bacteria

Table 3. XPS Binding Energies (eV) and Atomic Concentrations (%) at the Surface of PMMA, PMMA/TPAB, PMMA/CTAB, and PMMA/DODAB Hybrid Films before and after Leaching[a]

		C 1s					O 1s	
	Br^- (Br $3d_{5/2}$)	C−H	CR_4	O−CH_3	−O−C=O	$(NR4)^+$ (N 1s)	−O−C=O	−O−CH_3
bindingenergy (eV)	67.1	285.0	285.8	286.9	289.0	402.3	532.3	533.0
	% atomic composition							
film	Br 3d5	C 1s	C 1s	C 1s	C 1s	N 1s	O 1s	O 1s	Σ^v
PMMA		28.52	17.92	12.87	14.28		13.13	13.02	100.01
+ TPAB	0.41	29.83	18.20	13.15	13.38	0.46	12.24	12.15	99.82
+ CTAB	1.01	41.42	15.26	11.43	10.83	1.18	9.34	9.23	99.7
+ DODAB	1.72	72.23	12.44	3.51	3.37	1.69	2.53	2.51	100
PMMA[b]		28.64	17.50	13.18	14.03		13.34	12.88	99.57
+ TPAB[b]	0	27.88	19.78	13.39	13.56	0	12.75	12.39	99.75
+ CTAB[b]	0	28.36	19.24	13.93	12.97	0.15	12.72	11.74	99.26
+ DODAB[b]	0	49.00	13.71	8.69	8.62	0.65	10.16	7.3	98.23

[a] Final QAC and PMMA concentrations in the chloroformic solution spin-coated on the silicon wafers was 10 mg/mL PMMA and 3.8 mM QAC. [b] QACs leaching was induced by immersing the films in 3 mL water with replacement of this same water volume at least three times over 12 h. Thereafter, the films were dried under vacuum before XPS analysis. This procedure was done for films marked with an asterisk. [v] Concentration balances (Σ) smaller than 100% were due to weak peaks of unidentified contaminants in the N 1s and O 1s region.

in several other instances.[14,24] Interestingly, Gram-negative bacteria were reported to change their outer envelope composition as a defense mechanism in response to QACs.[36] Furthermore, *Pseudomonas* exhibited a much higher level of resistance toward antimicrobial agents based on QACs than *Staphylococcus* spp. or *E. coli*.[37] In fact, the high resistance of *Pseudomonas aeruginosa* to antimicrobial agents has been well-documented and reviewed in the literature.[38]

Adsorption isotherms for CTAB or DODAB onto *Candida albicans* cells showed that limiting adsorption was 7.8×10^9 and 3.7×10^9 molecules/cell for CTAB and DODAB, respectively.[39] Therefore, doubling the dose of the active quaternary ammonium with CTAB in comparison to DODAB explained its higher antimicrobial efficacy in dispersion. In fact, behind this higher CTAB potency to interact with membranes of bacterial cells was its more favorable hydrophobic−hydrophilic balance and molecular architecture. Whereas both CTAB and DODAB revealed their ability to reach bacteria in suspension by leaching from the films upon exposure to water, only DODAB preserved a sustained ability of killing bacteria upon contact with the film surface (Figures 2 and 5 and Table 3). Determination of intrinsic activity for TPAB alone showed its complete lack of bioactivity (Figure 4), consistently with the observed lack of bioactivity for PMMA/TPAB films (Figure 4) and with the literature on antimicrobial activity for QACs: those with alkyl radical R < 6C do not possess noticeable antimicrobial efficiency in solution.[40]

In conclusion, hybrid PMMA/QAC films showed antimicrobial properties when the QAC was DODAB or CTAB, in contrast with TPAB. This suggested that exposure of quaternary ammonium moieties to film surface and molecular architecture of the QAC were important to determine antimicrobial action. The unfavorable hydrophobic−hydrophilic balance of TPAB hampered its location at interfaces such as film−air, film−water, air−water, or even bacteria−water and imparted to this molecule complete absence of antimicrobial property. The higher antimicrobial activity of CTAB when compared to the one exhibited by DODAB was ascribed to its favorable hydrophobic−hydrophilic balance plus its higher ability to diffuse from the films as evidenced by surface tension reduction at the air−water interface and occurrence of an inhibition zone of bacterial growth in agar plates. The approach described in this work for mechanical immobilization of the QACs such as DODAB and CTAB on polymeric materials has potential applications in the pharmaceutical, medical, food or biotechnological industries where different needs may be satisfied by the different behavior of DODAB and CTAB. When complete release of the antimicrobial agent is required, CTAB may be used. If a more sustained action at the surface of the material is needed, DODAB may be the best choice. TPAB did not show antimicrobial properties neither in dispersion nor hybridized with the PMMA films.

■ ASSOCIATED CONTENT

Supporting Information. All XPS spectra supporting Table 3 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org/.

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