Biomaterials Science

PAPER

Cite this: *Biomater. Sci.*, 2016, **4**, 299

High durability and low toxicity antimicrobial coatings fabricated by quaternary ammonium silane copolymers†

Hairui Li,[a,b] Hongqian Bao,[b] Ke Xin Bok,[a] Chi-Ying Lee,[b] Bo Li,[b] Melvin T. Zin[b,c] and Lifeng Kang*[a]

Received 29th August 2015,
Accepted 25th October 2015

DOI: 10.1039/c5bm00353a

www.rsc.org/biomaterialsscience

Adhesion and subsequent growth of microorganisms on material surfaces are a major concern in many biomedical applications. Currently, various polymers are immobilized on material surfaces to prevent microbial colonization. However, there are several challenges with regard to the coating materials, including their inability to kill microorganisms, complexity of surface grafting, limited durability and toxicity towards humans. To address these challenges, we synthesize a novel quaternary ammonium silane (QAS) antimicrobial copolymer to confer the antimicrobial effect *via* a simple thermal-curing coating process. The QAS copolymers were less toxic to 3 human cell lines than a commercial antimicrobial QAS monomeric agent, namely, dimethyloctadecyl[3-(trimethoxysilyl) propyl]ammonium chloride (DTPAC). Moreover, the QAS coatings demonstrated superior antimicrobial efficacy and durability than those of the DTPAC coatings. In conclusion, the novel QAS copolymers are useful to prevent substrates from microbial infections, yet with low toxicity to humans and long durability. In addition, the synthetic process is potentially scalable for industrial applications.

Introduction

Adhesion and subsequent growth of microorganisms to form biofilms are of great concern in various areas, such as biomedical devices, healthcare applications, water purification systems, dental surgery equipment, food packaging, textiles, household sanitation, *etc*.[1,2] They can cause contamination of products and corrosion. Microbial colonization of biomaterials can also lead to systemic and implant-associated infections, the incidence of which is expected to increase with the expansion of new biomedical devices.[3] It was reported that microorganisms in biofilms are more resistant to disinfection at an extent of up to 1000 times compared to free-floating microorganisms.[4] Therefore, it is imperative that microbial growth is more efficiently inhibited in the early stage of microbial adhesion and proliferation.[5]

In recent years, antimicrobial polymers have attracted considerable interest from both academia and industry, owing to their advantages over small molecular biocides, being non-volatile, with increased stability, low toxicity, minimal permeability through skin and the potential to maintain long-term activity.[6] Researchers have tried to immobilize surfaces with polymers to prevent the formation of biofilms, thus avoiding the spread of diseases and material deterioration. The reported techniques to attach polymers to surfaces include chemical grafting techniques, layer-by-layer and surface-initiated polymerization.[7–9] Different from biocide impregnated surfaces, the grafting of antimicrobial polymers can produce non-leachable antimicrobial surfaces which can remain permanently antimicrobial.[6]

Highly hydrophilic polymers have been grafted to biomaterials to prevent biofilm formation by repelling microbes and preventing their attachment. Such polymers include poly(ethylene glycol) derivatives and poly(ethylene oxide), both of which display an exclusion volume effect which renders them capable of resisting non-specific protein adsorption and cell adhesion.[10] However, these polymer coatings are anti-adhesion based and don't have any biocidal activity. Microorganisms may be introduced into the patient during implantation procedures and the failure of these polymers to eliminate them can result in implant failure.[11]

The antimicrobial surfaces can be further modified to kill microbes in the vicinity by conjugating polymers with agents such as antibiotics or antimicrobial peptides or complexed with silver.[12–16] Wach *et al.* synthesized a hybrid molecule comprising a poly(ethylene glycol) chain with an anachelin chromophore at one end for the functionalization of surfaces

[a]*Department of Pharmacy, National University of Singapore, 18 Science Drive 4, Singapore 117543. E-mail: lkang@nus.edu.sg; Fax: +65 6779 1554; Tel: +65 6516 7519*
[b]*3M Innovation Singapore, 100 Woodlands Avenue, Singapore 738205*
[c]*School of Materials Science and Engineering, Nanyang Technological University, Block N4.1, Nanyang Avenue, Singapore 639798*
†Electronic supplementary information (ESI) available. See DOI: 10.1039/c5bm00353a

and an antibiotic, vancomycin at the other end responsible for antimicrobial activity.[17] Penicillin has also been tethered to different surfaces by being attached to poly(2-hydroxyethyl methacrylate) chains and poly(ethylene glycol) chains. But the antibiotics may be partially lost due to hydrolysis.[18–20] Ramstedt *et al.* reported the synthesis of poly(3-sulfopropyl-methacrylate) brushes onto gold and Si/SiO_2 surfaces by transfer radical polymerization, and the polymer brushes can be easily loaded with silver ions to exert the antibacterial activity.[13] Silver nanoparticles were loaded on decorated cellulose filter papers grafted with poly(*tert*-butyl acrylate) polymers, showing excellent antibacterial properties against *E. coli*.[21] However, the bactericidal action of silver relies to a certain extent on the leaching of the ions, which may cause certain environmental issues. An alternative way to create contact-active surfaces is to immobilize polymers conjugated with antimicrobial proteins (AMPs).[16] AMPs have been of great interest to study in recent years because they have a low propensity for developing microbial resistance.[14] Covalent immobilization of AMP onto the surface can increase their long-term stability while decreasing their cytotoxicity associated with higher concentrations of soluble peptides when compared to leach- or release-methodologies.[22,23] A common approach to covalently immobilize AMPs involves the use of functionalized resins such as poly(ethylene glycol) spacers or other polymeric 'brushes' with reactive groups suitable for peptide covalent conjugation. However, the antimicrobial activity of AMPs decreased after immobilization in many reported studies.[24–26] The immobilization parameters, such as peptide surface concentration, influence of the spacer (length and flexibility) or peptide orientation after immobilization, must be optimized to obtain efficient, safe, and long-lasting antibacterial coatings.[27] Furthermore, the cost and complexity of their synthesis and immobilization add up to the disadvantages of AMPs as well.

A third class of antimicrobial polymeric surfaces was created by immobilizing bactericidal polymer chains to kill the bacteria upon contact.[6] These polymers usually contain cationic groups, such as quaternary ammonium or phosphonium groups. Tiller *et al.* reported poly(vinyl-*N*-pyridinium bromide) modified surfaces exhibiting good antibacterial properties, but the complicated method may limit it in routine practices.[28] Lee *et al.* grafted an antibacterial quaternary ammonium polymer directly on the surface of the glass using atom transfer radical polymerization, but this approach lacks the ease of implementation as well.[29] On the other hand, quaternary ammonium silane (QAS) monomeric agents, such as trimethoxysilyl and trihydroxysilyl quaternary ammonium compounds, have been used to prepare antibacterial surfaces easily by covalent attachment to a surface through a reaction of the trimethoxysilyl groups with surface silanol groups.[30] These compounds are widely used as bacteriostatic and fungistatic treatments in human clothing and bedding, household areas, carpets and upholstery. QAS agents have the largest consumption volume as textile antimicrobials compared with triclosan and silver.[31] They are also used as material preservatives in the manufacturing of paints, coatings, and concrete. However, despite the widespread use of QAS agents, severe toxicity has been observed with regard to skin and eye irritations[32] and contact dermatitis was also reported with the QAS coating.[33,34] Besides, the *in situ* condensation coating on the surface was supposed to form a monomolecular layer and it may not be resistant to abrasion. The antimicrobial activity is lost after the surface layer is worn off.

To address these problems, we have synthesized novel QAS antimicrobial copolymers *via* a simple free radical addition reaction and an abrasion-resistant antimicrobial surface can be further prepared by a simple and easy thermal-curing process. The synthesized copolymers were found to be less toxic to human cell lines than a commercial antimicrobial QAS monomeric agent, namely, dimethyloctadecyl[3-(trimethoxysilyl)propyl]-ammonium chloride (DTPAC). The QAS antimicrobial copolymer coatings demonstrated a broader antimicrobial activity and a better durability than those formed by DTPAC. We envision that, with a low toxicity and enhanced durability, the new QAS copolymers are appealing and represent a better substitute for the current monomeric QAS coatings.

Materials and methods

Materials

The monomers used for polymer synthesis are [3-(methacryloylamino)propyl]trimethyl ammonium chloride (MAPTAC, 50 wt% in H_2O, Sigma-Aldrich) and 3-trimethylsilylpropyl methacrylate (TMSPMA, Sigma-Aldrich). Potassium persulfate ($K_2S_2O_8$, Sigma-Aldrich) was employed as a free radical initiator for polymerization and acetone (Merck) was used to precipitate the synthesized polymers. A commercialized antimicrobial agent, DTPAC (42 wt% in methanol, Sigma-Aldrich) was used as a positive control in the antimicrobial test of coatings. Acetic acid (Merck) was used to prepare the acidic solution for QAS polymers' hydrolysis. Phosphate-buffered saline (PBS, pH 7.4) for the dilution of polymer solutions in the assays was prepared from 10× PBS (Vivantis, Malaysia). The bacteria were grown in nutrient broth (Neogen, USA) and on nutrient agar prepared from bacteriological agar (Neogen, USA). The fungus was grown in tryptone soya broth (Neogen, USA) and on tryptone soya agar (Neogen, USA). The human cells were grown in Dulbecco's modified eagle medium (DMEM, Life Technologies, Singapore) supplemented with 10% fetal bovine serum (FBS, Life Technologies, Singapore) and 1% penicillin–streptomycin solution (PAN-Biotech GmbH, Germany). Adherent cells were detached from culture surfaces *via* trypsin prepared from 10× trypsin-EDTA (PAA Laboratories, Austria). Thiazolyl blue tetrazolium bromide (MTT, 98%, MP Biomedicals, Singapore) and dimethyl sulphoxide (DMSO, MP Biomedicals, Singapore) were reagents for the MTT cytotoxicity assay. All other chemicals were obtained from Sigma-Aldrich and used as received.

Preparation and characterization of QAS copolymers and coatings

QAS copolymers PMT-5% and PMT-10% were synthesized from the monomers MAPTAC and TMSPMA in the presence of

1 wt% $K_2S_2O_8$. PMT-5% was synthesized from the monomer weight ratio of 95% MAPTAC (19.0 g) to 5% TMSPMA (0.5 g) while PMT-10% was synthesized from 90% MAPTAC (18.0 g) to 10% TMSPMA (1.0 g). Homopolymer PMAPTAC was synthesized from MAPTAC (20.0 g) in the presence of 1 wt% $K_2S_2O_8$. The reaction proceeded at 70 °C with 400 rpm stirring speed for 1 hour. Thereafter, 15 mL of water was added to terminate the reaction and decrease the viscosity of the mixture. The resulting copolymers and homopolymers were precipitated against acetone (3 times) and vacuum dried at 50 °C for 48 hours.

Glass slides (25.4 × 76.2 mm, Continental Laboratory Products, USA) were used as substrates to study the coating process of PMT-5% and PMT-10%. Prior to coating, the glass slides were immersed in 10% sodium hydroxide for 1 hour, thoroughly rinsed with water and cleaned with 96% ethanol before air drying. A pH 2.5 acetic acid solution was used to hydrolyze the silyl ether to the corresponding reactive silanol groups in copolymers. Acetic acid was added dropwise to water until the desired pH was reflected by the pH meter. 10 mg mL^{-1} of polymer in acid solution was transferred to the substrate surface by 29 μL cm^{-2} and spread evenly using a pipette. Crosslinking of silanol groups to form a siloxane linkage was initiated by drying the substrate at 130 °C for 30 minutes.

A Spectrum 100 Fourier transform infrared (FTIR) spectrophotometer (PerkinElmer, USA) with an attenuated total reflectance (ATR) set-up was used to monitor the polymer synthesis, hydrolysis and crosslinking reactions. Infrared spectra were collected between 4000 and 650 cm^{-1} at a resolution of 4 cm^{-1}. The water contact angle was measured with a static contact angle measurement device (Attension, BiolinScientific). The machine was equipped with a digital camera, automatic liquid dispensers, and a sample stage allowing hands-free contact angle measurement *via* automated placement of a drop of water. The drop shape is captured automatically and then analyzed *via* OneAttension software to determine the static contact angle. After coating, the glass slides were subjected to an abrasion test by using a Taber Linear Abraser 5750 instrument with a vertical load of 1 kg, and 10 cycles were performed with a magnitude of 1 inch. The characteristic peak absorbance from ATR-FTIR examination of coating before and after abrasion at the same location was used to calculate the retention of coating on the glass slides. The retention of coating was expressed as the peak height of the characteristic peaks before abrasion divided by that of after abrasion.

Antimicrobial assays of QAS copolymers and coatings

15% glycerol cell stocks of *Staphylococcus aureus* (*S. aureus*, ATCC 6538P, Oxoid, Singapore), *Escherichia coli* (*E. coli*, ATCC 8739, Oxoid, Singapore) and *Candida albicans* (*C. albicans*, ATCC 2091, MicroBiologics, France) were prepared and divided into 1 mL aliquots and frozen at −80 °C. Bacterial glycerol stocks were thawed and diluted 1 : 10 in PBS. 0.1 mL of the diluted stock was made up to 15 mL with nutrient broth and grown at 37 °C and 200 rpm. Fungal stocks were thawed and diluted 1 : 100 in PBS. 0.1 mL of the diluted stock was made up to 15 mL with tryptone soya broth and grown at 28 °C and 200 rpm.

The minimum inhibitory concentration (MIC) assay was carried out *via* the broth microdilution method using sterile 96-well flat-bottomed microplates (Costar, Corning, USA). *S. aureus* (Gram-positive), *E. coli* (Gram-negative) and *C. albicans* (fungus) were grown to their exponential phase and appropriately diluted to achieve the inoculum size of approximately 10^5 colony forming units (CFU) mL^{-1} in each well. The MAPTAC homopolymer (PMAPTAC), PMT-5% and PMT-10% were dissolved in sterile PBS to get a concentration of 5 mg mL^{-1}. Solutions were sterilized *via* filtration with a 0.20 μm polyethersulfone filter (Sartorius Stedim Biotech, Germany) and serially diluted with PBS to achieve a broad range of polymer concentrations. A 20 μL polymer solution with different concentrations was added to 180 μL inoculum in each well. The microplate was incubated at 37 °C (for bacteria)/28 °C (for fungus) with shaking at 200 rpm for 24 hours before being assayed at 600 nm using a microplate reader (Tecan, Switzerland). The MIC is defined as the lowest polymer concentration at which there is no increase in optical density. Sterile broth, sterile polymer solutions of various concentrations dissolved in broth and broth containing microorganism alone were used as controls. After the microplate used in the MIC assay was incubated for 24 hours, minimum bactericidal/fungicidal concentration (MBC/MFC) determination was performed for concentrations at and above MIC. Briefly, a 100 μL aliquot was removed from each well showing no growth except wells containing the sterility controls. Each aliquot was plated onto nutrient agar in duplicate and incubated at 37 °C for 24 hours (or plated onto tryptone soya agar plates and incubated at 28 °C for 3 days for *C. albicans*) before conducting a colony count. MBC/MFC is defined as the lowest concentration that achieves a ≥99.9% or a three-log reduction in the initial MIC inoculum within 24 hours. This was determined by calculating the maximum allowable number of colonies per plate. The rejection value (CFU per plate) was expressed as initial inoculum (CFU mL^{-1}) × 0.1 mL × 0.1%.

The test for antimicrobial activity of the polymers' coating was conducted on two substrates, glass cover slips (Menzel-Gläser, Germany) as well as a zirconia and silica containing nanocomposite dental restorative, Lava™ Ultimate (3M™ ESPE).[35,36] Before coating, the glass cover slips were treated in the same way as glass slides previously described in the methods part. Lava restorative was firstly polished with 3M Trizact™ abrasive to give a fresh surface, and cleaned with DI water and 96% ethanol before air drying. The treated surfaces of the cover slips (22 × 22 mm) and Lava restorative (22 mm × 18 mm) were covered with acidified PMT-5%, PMT-10% and DTPAC solutions (10 mg mL^{-1}) at 29 μL cm^{-2}, respectively, and dried at 130 °C for 30 minutes. For antimicrobial assessment of coatings, the microorganisms were grown to their exponential phase and appropriately diluted to achieve the standard inoculum size between 9 × 10^5 and 10^6 CFU mL^{-1}. The coated substrates were placed in a sterile Petri dish and an appropriate amount of bacterial inoculum was transferred

onto the surface, and then it was covered with a plastic film (18 × 18 mm for a cover slip and 18 × 15 mm for Lava restorative) to achieve the inoculum amount of at least 10^4 cells cm^{-2}. Uncoated substrates were used as controls. Inoculated microorganisms were recovered by removing the film-covered substrates and placing them into 50 mL Falcon tubes containing 10 mL of PBS. The tubes were vortexed for 1 minute before serially diluting the samples in PBS. A viable count was conducted using the spread plate method after appropriate dilution. Bacteria recovery was carried out after incubation at 37 °C under a humidified atmosphere for 24 hours while incubation temperature of 28 °C was used for *C. albicans*. The unit number of viable microbes was obtained from the counts and the colonies calculated according to the following equation:

$$N = C \times D \times V/A$$

where N: Number of viable microbes per 1 cm^2. C: Count of colonies. If $C < 1$, C is taken as 1. D: Dilution factor. V: Volume of recovery solution. A: Area of cover film (cm^2). The antimicrobial activity was expressed as log reduction number of the microorganisms after 24 hours compared with control.

Field emission scanning electron microscopy (FE-SEM) analysis

FE-SEM was applied to analyze the antimicrobial mechanism of the QAS copolymer by investigating the bacterial morphology change. *S. aureus* was adjusted to a concentration of 10^9 cells per mL before incubating with or without PMT-10% (to a final concentration of 2 × MIC) for 0.5 h, and the obtained solutions were centrifuged at 4000 rpm for 5 min. The precipitated bacteria were rinsed twice with PBS, immobilized with 2.5% glutaraldehyde for 60 min, and again rinsed with water twice. Cell dehydration was performed using a series of ethanol/water solutions (35, 50, 70, 90, 95, and 100%), and the final dehydrated sample was transferred onto a carbon tape and dried at room temperature for 2 days. Platinum sputtering was applied to coat the dry samples before FE-SEM imaging (JSM-6700F, JEOL, Japan).

Cytotoxicity assay

The cytotoxicity of the polymers was evaluated *via* the determination of mitochondrial succinate dehydrogenase activity using an MTT assay with human dermal fibroblasts (HDF), human adult low calcium high temperature (HaCaT) keratinocytes and human embryonic kidney 293 (HEK293) cells. PMAPTAC, PMT-5%, PMT-10% and DTPAC were dissolved in PBS with a concentration of 2.5 mg mL^{-1}, sterilized *via* filtration and further diluted with PBS to the desired concentrations. Cells were seeded at a density of 10^4 cells per well in 96-well flat-bottomed microplates (Costar, Corning) and incubated with DMEM culture medium (supplemented with 10% FBS and 1% penicillin–streptomycin solution) for 24 hours. Thereafter, the culture medium was replaced with 180 μL of fresh medium and 20 μL of polymer solution in each well. The cells were incubated with the different concentrations of polymers and DTPAC for 24 hours at 37 °C, 5% CO_2 and 95% relative humidity before removal of the medium. 200 μL of fresh medium and 20 μL of filtered MTT solution (5 mg mL^{-1} in PBS) were further added and the cells were incubated for another 4 hours at 37 °C. Then, the supernatant was removed by aspiration, and resultant formazan crystals were dissolved in 150 μL of DMSO, followed by shaking for 5 min. The absorbance at 595 nm (A_{595}) was measured using a microplate reader (Tecan, Switzerland). Wells containing DMSO alone were used as a blank, and cells treated with PBS alone were used as control for the assay. Relative cell viability was expressed as (A_{595} sample − A_{595} DMSO)/(A_{595} control − A_{595} DMSO) × 100%. For each sample, the final absorbance was the average of those measured from six wells in parallel. Since DTPAC was provided in 42 wt% in methanol, the toxicity of methanol was also tested against the three kinds of cells after proper dilution. Methanol with the same concentrations in tested DTPAC solutions showed no significant difference with PBS control.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance followed by the Tukey's *post-hoc* test. The difference was considered to be significant at p-value < 0.05.

Results

Fabrication and characterization of QAS copolymers and coatings

Fig. 1 illustrates the reaction scheme for the synthesis of QAS copolymers from two monomers, namely, [3-(methacryloylamino)propyl]trimethyl ammonium chloride (MAPTAC) and 3-trimethylsilylpropyl methacrylate (TMSPMA), with two different weight ratios. The synthesized copolymers, named PMT-5% and PMT-10%, derive their antimicrobial properties from the cationic quaternary ammonium group in MAPTAC while utilizing the silane functionality in TMSPMA as an anchor. $K_2S_2O_8$ functions as a free radical initiator for

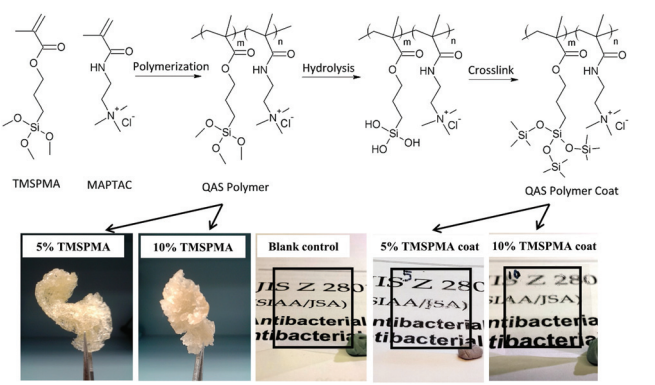


Fig. 1 Synthesis scheme of QAS copolymers and coatings. The physical appearances of synthesized QAS copolymers of PMT-5% (5% TMSPMA) and PMT-10% (10% TMSPMA) are shown, with the optical transparency of QAS copolymer coatings in comparison to the control.

polymerization which proceeds *via* a free radical addition reaction involving the saturation of the α,β-unsaturated amide in MAPTAC and the α,β-unsaturated ester in TMSPMA. The unreactive silyl ether acts as a protective group for the silane functionality during this reaction. Subsequently, it is activated *via* hydrolysis in acidic medium which was achieved by dissolving the polymers in pH 2.5 acetic acid solutions. This yielded highly reactive silanol groups which readily undergo dehydration reactions involving the loss of water. Drying the acidified polymer solution under high temperatures of 130 °C gave rise to the formation of siloxane crosslinking amongst polymers as well as between polymers and the substrate surfaces where reactive groups were present. The synthesized copolymers appear yellow (Fig. 1) which is typical of the physical appearance of most QACs.[37] Resultant alterations to the color of QAC-treated surfaces are a disadvantage limiting the applications of these polymers. In contrast, the copolymer coatings formed by the QAS copolymers PMT-5% and PMT-10% appear transparent with minimal haziness observed in comparison to the uncoated cover slip even when coated at high concentrations of 10 mg mL^{-1}. As the polymer coating is intended for treating surfaces, it is desirable for the coating to have negligible effects on the appearance of treated surfaces. Hence, the transparency of the polymer coating renders it to be suitable for a broad array of potential surface applications.

The ATR-FTIR spectra were used to monitor the copolymer synthesis, extent of hydrolysis and condensation reactions undergone by the copolymers during the coating process (Fig. 2). Characteristic stretches of the Si–O bond in alkoxysilane compounds appear as strong bands at 1090 cm^{-1} and sharp bands around 2840 cm^{-1} and in the copolymers' spectra, that is 2850 cm^{-1} in PMT-5%'s spectrum and 2842 cm^{-1} in PMT-10%'s spectrum. The Si–O bond of silanol groups showed absorption as a single band at 915 cm^{-1}. These peaks confirmed the successful polymerization of the copolymers. Changes to the bands characteristic of the Si–O stretching vibration of the silyl ether supported the inference that copolymer hydrolysis had occurred. Specifically, the disappearance of the sharp band at 2850 cm^{-1} in PMT-5%'s spectra and 2842 cm^{-1} in PMT-10%'s spectra can be observed. There was also a discernible reduction in the intensity of the strong band located at 1090 cm^{-1} in the copolymers' spectra after coating. As both bands were associated with the Si–O stretching vibration of the silyl ether group, these observations were consistent with the occurrence of hydrolysis. During the drying process, the formation of siloxane crosslink in the copolymer coatings restrained the stretching vibration of the carbonyl group in the ester functionality, causing the carbonyl stretch in the 1700 cm^{-1} region to disappear. Evidence for the successful crosslinking in the current study was thus shown by the disappearance of the carbonyl bands at 1718 cm^{-1} in PMT-10%'s spectra (Fig. 2B). The reduction of the Si–O bond of silanol groups at 915 cm^{-1} indicated the existence of conversion of silanol groups to siloxane crosslinks during the drying process.

The thermal degradation of QAS polymers was investigated by using thermogravimetric analysis (Fig. S1†). The initial

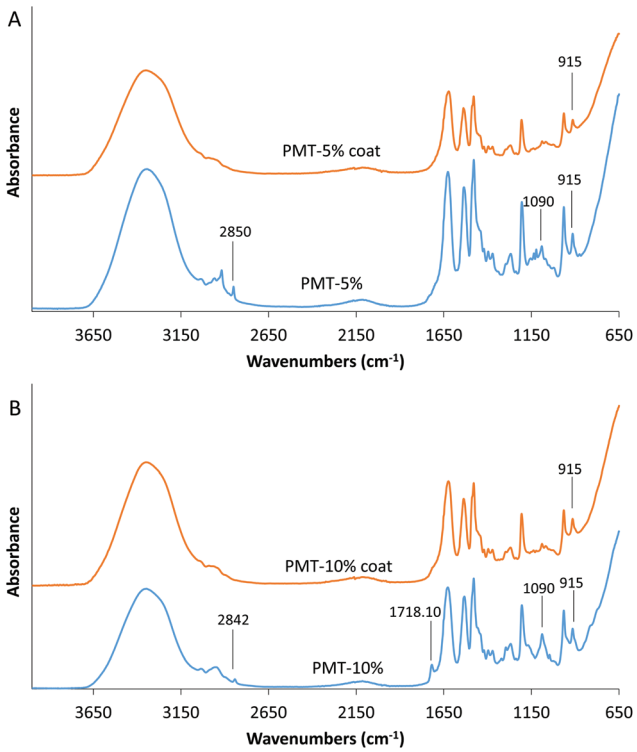


Fig. 2 ATR-FTIR spectra of: (A) PMT-5% copolymer and coating; (B) PMT-10% copolymer and coating.

degradation temperature of PMAPTMC, PMT-5% and PMT-10% was found to be in the range of 292 °C to 299 °C. The high degradation temperature indicated high thermal stability of the synthesized polymers.

The surface energy and hydrophobicity of QAS coatings were evaluated by measuring the static contact angles. Fig. 3 shows the contact angles of PMT-5%, PMT-10% and the commercial antimicrobial agent DTPAC coating against a glass control, which can be considered as a hydrophilic surface due to the low value (26.1°). The QAS coatings are more hydrophobic and have higher contact angle values than the glass control. Furthermore, the QAS copolymer PMT-5% and PMT-10% coated glasses have even higher contact angles (107.4° and 104.7°) than the DTPAC coated glass (80.9°).

The durability of the QAS copolymer coatings was evaluated by an abrasion test combined with ATR-FTIR analysis. As a control, coatings formed by the MAPTAC homopolymer (PMAPTAC) and the commercial QAS monomeric antimicrobial agent, DTPAC were tested. As shown in Fig. 4, the PMAPTAC coating shows a low retention of less than 20%. The PMT-5% and PMT-10% coatings showed high retention up to 81.3% and 92.5%, respectively. It is noted that the coating retention of the monomeric QAS agent DTPAC was 42.5%, almost half of PMT coatings.

Antimicrobial efficacy of QAS in solutions

The antimicrobial activities of the synthesized polymers together with DTPAC were tested against Gram-positive bac-

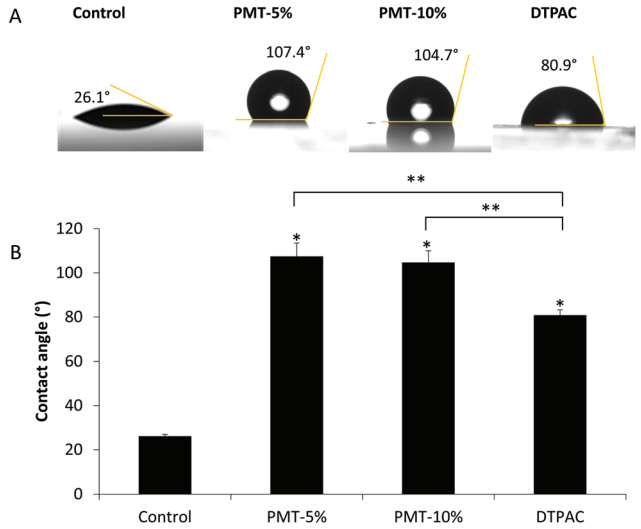


Fig. 3 (A) Water droplets on the surfaces of uncoated and coated glass. (B) Contact angles of the surfaces of uncoated and coated glass. Data represents the standard deviation of at least three tested samples. p-value < 0.05 indicates statistically significant, and * shows significance between coated samples and control, while ** shows significance between different coated samples.

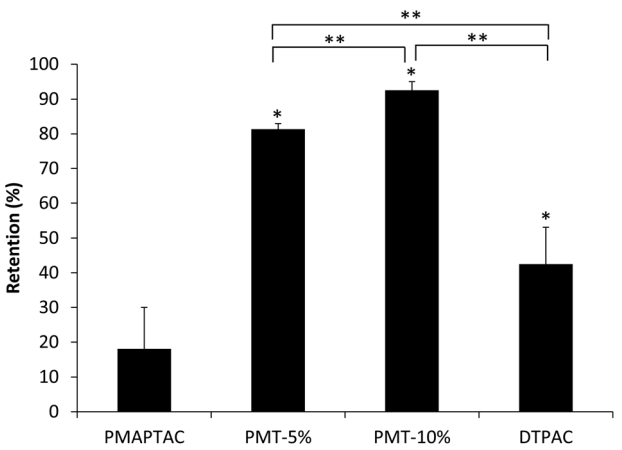
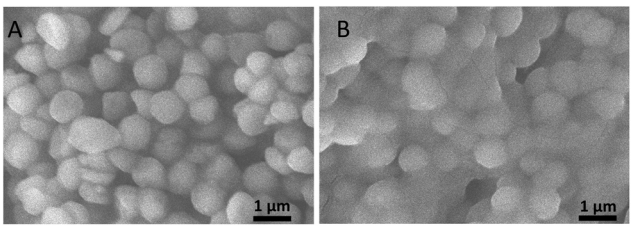


Fig. 4 Retention of characteristic peaks of antimicrobial coatings after the abrasion test. Data represents the standard deviation of three tested samples. p-value < 0.05 indicates statistically significant, and * shows significance between the QAS coated samples and PMAPTAC coated samples, while ** shows significance between different QAS coated samples.



C

Microorganisms		PMAPTAC	PMT-5%	PMT-10%	DTPAC
S. aureus	MIC	62.5	62.5	62.5	3.9
	MBC	250	125	125	3.9
E. coli	MIC	62.5	125	62.5	62.5
	MBC	62.5	125	62.5	62.5
C. albicans	MIC	> 500	> 500	> 500	15.6
	MFC	> 500	> 500	> 500	62.5

Fig. 5 SEM images of *S. aureus* before (A) and after (B) 30 min treatment with PMT-10% at 2 × MIC. (C) MIC and MBC/MFC against *E. coli*, *S. aureus* and *C. albicans* at an inoculum size of approximately 10^5 CFU mL^{-1}. The unit of the MIC and MBC/MFC values is μg mL^{-1}.

teria *Staphylococcus aureus* (*S. aureus*), Gram-negative bacteria *Escherichia coli* (*E. coli*) and fungus *Candida albicans* (*C. albicans*). As shown in Fig. 5C, the synthesized polymers had MICs in the range of 62.5 to 125 μg mL^{-1} against *E. coli* and *S. aureus* and >500 μg mL^{-1} against *C. albicans*. A comparison of the antibacterial activity of PMT-5% and PMT-10% to that of PMAPTAC revealed a similar minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 62.5 μg mL^{-1} with the sole exception of PMT-5% displaying slightly lower activity against *E. coli* at 125 μg mL^{-1}. The similar MIC of PMAPTAC with that of PMT-5% and PMT-10% provided evidence that the copolymerization of the quaternary ammonium monomer (MAPTAC) with TMSPMA did not affect its antimicrobial activity. Another notable aspect of the QAS copolymers' antimicrobial activity is their comparable efficacy against both *S. aureus* and *E. coli*, as evidenced by their similar MIC values. PMT-5% requires only a concentration twice its MIC against *S. aureus* to inhibit the growth of *E. coli* while PMT-10% inhibits the growth of both bacteria at the same concentration. On the other hand, although the monomeric QAS agent DTPAC has a lower MIC value against *S. aureus* than that of the synthesized QAS polymers, their MIC values against *E. coli* are comparable. In addition, the MBC : MIC ratios for all the QAS compounds and bacteria are ≤4, indicating the antimicrobial mechanism of action is bactericidal rather than bacteriostatic.[38] However, the copolymers were less effective against the fungus *C. albicans* as they failed to inhibit its growth even at higher concentrations of >500 μg mL^{-1}, while the monomeric QAC agent DTPAC was effective against *C. albicans* with a MIC value of 15.6 μg mL^{-1}.

As mentioned earlier, the QAS copolymers are able to disrupt the cytoplasmic membrane of bacteria *via* the interaction between the cationic quaternary ammonium group of the polymers and the anionic bacterial cell surfaces. This membrane lytic mechanism was further investigated by using scanning electron microscopy (SEM) to characterize the morphological change of *S. aureus* cells after incubation with the QAS copolymer. Since there were not many solid residues that could be found after mixing polymers with bacteria at the same concentration (10^5 cells per mL) with the MIC test, a higher bacteria concentration (10^9 cells per mL) was used for SEM testing. As another evidence to show the efficiency of QAS copolymers, the cloudy bacterial solution became semi-trans-

parent after the incorporation of PMT-10%, and a significant reduction in the amounts of bacterial residue pellets were observed after the centrifugation. The SEM images of *S. aureus* with and without treatment were shown in Fig. 5A and B respectively. It can be found that survival *S. aureus* treated with an above MIC concentration of PMT-10% had obvious membrane blebbing and was covered by the mixture of QAS copolymers and sticky intracellular constituents.

Cytotoxicity assay of QAS in solutions

The cytotoxicity of QAS polymers was tested against HDF, HaCaT keratinocytes and HEK293 cell lines. Human cells' viability predictably decreased with increasing concentration of PMAPTAC, PMT-5%, PMT-10% and DTPAC (Fig. 6) as quaternary ammonium polymers possess membrane-disruption ability.[39] At a polymer concentration of 62.5 μg mL^{-1}, HDF viability remained reasonably high for PMAPTAC (51.8%), PMT-5% (57.3%) and PMT-10% (52.3%) but very low for DTPAC (6.6%) (Fig. 6A). Results of HaCaT keratinocyte viability were generally consistent with the HDF assay results for both PMAPTAC (55.7%) and PMT-10% (54.5%) but a slightly lower viability was observed for PMT-5% (38.4%) and an even lower viability for DTPAC (7.2%) (Fig. 6B). For HEK293, cell viability was 57.7%, 50.0% and 63.2% for PMAPTAC, PMT-5% and PMT-10%, respectively, while the cells were almost all dead (2.4%) for DTPAC at a concentration of 62.5 μg mL^{-1} (Fig. 6C). It seemed that PMAPTAC and PMT-10 resulted in higher cell viability than control at concentration of 3.91 μg ml^{-1} in Fig. 6C, but the difference is not significant from the ANOVA test followed by the Tukey's *post-hoc* test. HEK cells are suspension cells thus have limited adherence to the bottom of a 96-well plate. So variation may arise from the culture medium removal and MTT solution removal steps.

Antimicrobial efficacy of QAS coatings

We further tested the antimicrobial activity of the QAS copolymers when they were coated on glass substrates as well as on a commercial dental restorative material, 3M Lava Ultimate. In comparison, blank glass/Lava substrates and coatings formed by the monomeric QAS antimicrobial agent, DTPAC were used as controls.

As shown in Fig. 7, antimicrobial activities of all the QAS coatings, including PMT-5%, PMT-10% and DTPAC coatings, were calculated to be 4.0 against *S. aureus* and 4.3 against *E. coli* after 24 hours, respectively. In fact, there were no viable bacteria recovered from all the QAS coated samples after 24 hours. On the other hand, the antimicrobial activities of PMT-5%, PMT-10% and DTPAC coatings against *C. albicans* were 2.0, 2.1 and 0.2, respectively. According to the test for measuring the antimicrobial activity of surfaces, significant antimicrobial effectiveness is defined as antimicrobial activity ≥2.0 after 24 hours. Therefore, QAS copolymeric coatings showed significant antimicrobial effectiveness against all the three microorganisms, while DTPAC was only effective against *S. aureus* and *E. coli*, but not effective against *C. albicans*.

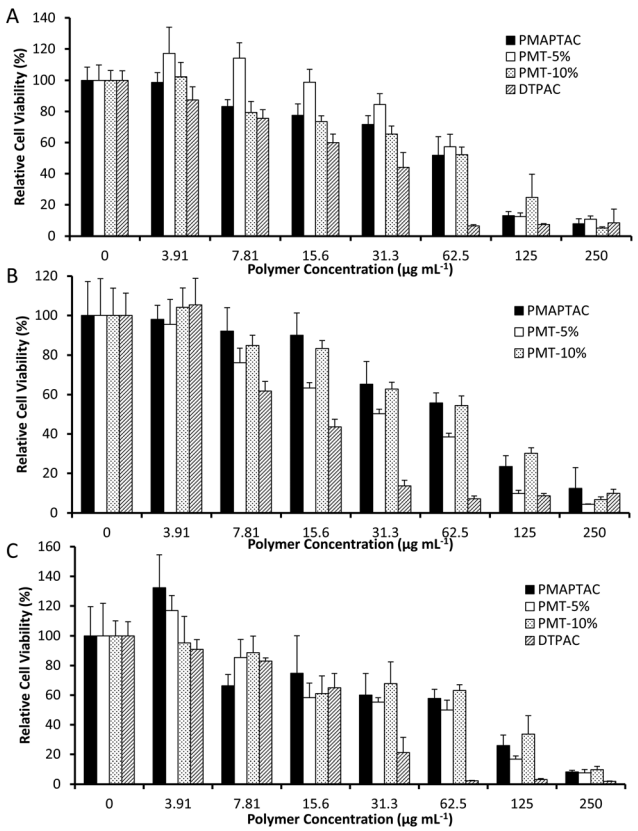
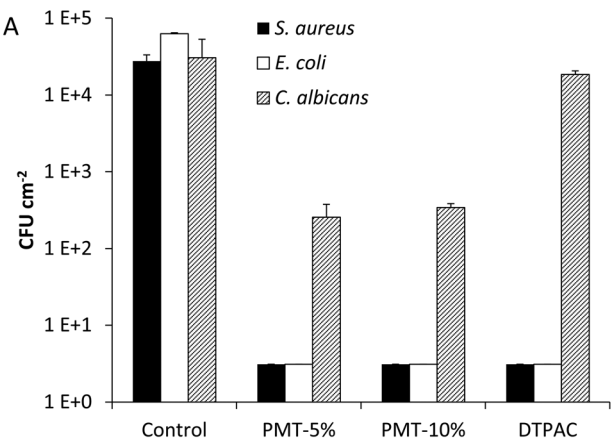


Fig. 6 Relative human cell viability after 24 hours exposure to different PMAPTAC, PMT-5% and PMT-10% concentrations: (A) HDF, (B) HaCaT keratinocytes, and (C) HEK 293 cells.

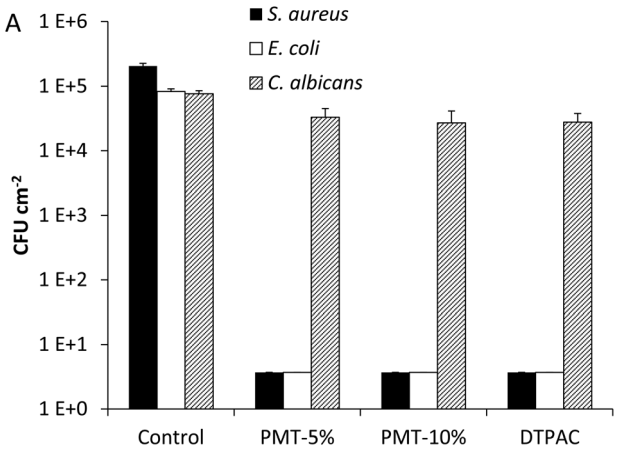


B

	S. aureus	*E.coli*	*C. albicans*
PMT-5%	4.0	4.3	2.1
PMT-10%	4.0	4.3	2.0
DTPAC	4.0	4.3	0.2

Fig. 7 Variable bacterial counts of QAS coatings against *S. aureus*, *E. coli* and *C. albicans* after 24 hours of exposure to coated glass cover slips (A) and the corresponding numerical values (B).

A



B

	S. aureus	*E.coli*	*C. albicans*
PMT-5%	4.7	4.3	0.36
PMT-10%	4.7	4.3	0.45
DTPAC	4.7	4.3	0.44

Fig. 8 Variable bacterial counts of QAS coatings against *S. aureus*, *E. coli* and *C. albicans* after 24 hours of exposure to coated Lava restoratives (A) and the corresponding numerical values (B).

From Fig. 8, the antimicrobial activities of all QAS coatings on Lava restoratives against *S. aureus* and *E. coli* were found similar to those on glass substrates with excellent antimicrobial activities. However, their antimicrobial activity against *C. albicans* was not as effective as their activity against bacteria. The QAS coatings showed a significant reduction in the fungi number after 24 hours' incubation, but the log reduction is less than 2.

Discussion

Microbial contamination and subsequent biofilm formation are the major causes of infection, contamination, and product deterioration. Considering that it is very difficult to remove the biofilm after its formation, a useful strategy is to prevent biofilm formation before it starts. QAS agents can be used to coat various substrates to impart the products with antimicrobial properties to prevent microbial contamination.[30] To reduce the toxicity of the QAS monomeric agent while retaining its antimicrobial efficacy and coating simplicity, we designed the novel QAS copolymers from a quaternary ammonium containing monomer and a silane containing monomer. The polymerization process is simple, potentially easy to scale up. At the same time, the coating process of the copolymer to a surface can remain the same as that of QAS monomeric agents, which doesn't need complicated techniques.

The QAS polymer coatings were characterized by using ATR-FTIR and further confirmed by contact angle measurements. It was shown that the QAS copolymer coatings increased the hydrophobicity of the glass surfaces, with an even higher hydrophobicity than the coating formed by the QAS monomeric agent, namely, DTPAC. The hydrophobicity of the DTPAC coated surface may mainly arise from reorientation of the long alkyl groups (tail) of the silane compounds.[40,41] On the other hand, the new QAS copolymers increased the hydrophobicity of the coated surface even more, which may be due to the formation of the long alkyl chains of the synthesized polymers.

The hydrophobicity of substrate surfaces is one of the properties that can affect microbial adhesion. Effective low adhesion surfaces can be created by grafting polyhydrophilic polymers such as poly(ethylene glycol) derivatives and poly (ethylene oxide), both of which display an exclusion volume effect which renders them capable of resisting non-specific protein adsorption and cell adhesion.[10] However, it has also been reported that bacterial adhesion was reduced on the hydrophobic surface due to the weak binding energy at the interface between the bacterium and the hydrophobic surface.[42,43] A positive correlation between material surface hydrophobicity and the detachment of biofilm was also reported, and the attached cells on hydrophobic surfaces were easily removed by an increased flow or an air-bubble jet.[5,44] Actually, microorganisms themselves can be hydrophobic, hydrophilic, or both, resulting from the differences in gene expression, such as production of flagella and fimbriae. In general, hydrophobic organisms are often repelled by hydrophilic surfaces and *vice versa*. Although whether the hydrophobicity of the surface is preferable for bacteria adherence is quite controversial, a research group has shown that the more hydrophobic silica nanoparticles coated glass surface adhered much fewer tested bacteria, including *E. coli*, *S. aureus*, and *D. geothermalis*.[41] Thus, the new QAS polymer coated surfaces, with a more hydrophobic property, may repel bacteria even better than the DTPAC coated surface theoretically. However, hydrophobic surfaces alone are neither enough to protect the surface from bacterial adhesion nor to eradicate the contacted bacteria; the sporadically adhered bacteria will grow and form a bacterial deposit eventually. The QAS polymer coating when applied to surfaces is expected to affect the adhesion properties of microorganisms, due to increased hydrophobic properties of the long alkyl backbone chain. Moreover, it can directly destroy unicellular organisms through the quaternized nitrogen on the side chain.

Durability and non-leaching properties are critical factors for antimicrobial coatings to provide long-lasting antimicrobial performance without toxicity concerns. Abrasion tests showed that the QAS copolymer coatings became much more resistant to abrasion with TMSPMA containing siloxane linkages, which can anchor the substrate by covalent bonding other than physical attachment. The improved resistance of PMT-10% against abrasion over PMT-5% should be attributed to the higher TMSPMA content which provided more "anchors" with the coated substrates. Furthermore, the QAS copolymers also showed more resistant to abrasion than the QAS monomeric agent DTPAC. QAS monomeric agents presumably form a monolayer molecular coating, while QAS polymers can form a

more mobile coating with the polymer long-chains stretched out to the surroundings, thus having a higher durability.

The crucial characteristics of polymers intended for use as antimicrobial materials in household, industrial and clinical applications include not only the presence of antimicrobial activity but also the absence of toxicity to human cells. Differential cytotoxicity to microbial cells and human cells should invariably be addressed when evaluating the QAS copolymers for future *in vivo* applications. The cell viability at a polymer concentration of 62.5 μg mL^{-1} is of particular interest since this is the concentration at which the polymers exert their antimicrobial effect against both *S. aureus* and *E. coli* as shown by the MIC and MBC results (Fig. 5). The *in vitro* cytotoxicity assay demonstrated reasonably high cell viability at a polymer concentration of 62.5 μg mL^{-1} for our synthesized QAS polymers: HDF viability ranging from 51.8% to 57.3%, HaCaT viability ranging from 38.4% to 55.7%, and 50.0% to 63.2% for HEK293. Interpreting these results in the context of PMT-10%'s antimicrobial activity, only ≤0.1% of *S. Aureus* and *E. coli* survive while *ca.* 50% of HDF, HaCaT and HEK293 cells remain viable at 62.5 μg mL^{-1}. But for the monomeric QAS agent, DTPAC, the cell viability of the three cells is less than 8% at 62.5 μg mL^{-1}, a concentration at which DTPAC can kill more than 99.9% of both *S. aureus* and *E. coli*. These findings suggest that our synthesized QAS copolymers are less toxic to human cells than the commercial QAS monomeric agent, DTPAC. So the synthesized QAS copolymers are supposed to be safer than DTPAC during handling.

The MIC and MBC/MFC tests showed that the QAS copolymers are less effective than the commercial QAS monomeric agent, DTPAC, especially against fungus *C. albicans*. However, results from the MIC and MBC/MFC tests represent the QAS compounds' antimicrobial activity in solution and are not directly indicative of their properties upon coating.[6,16] Hence, antimicrobial testing with the QAS copolymer treated surfaces were carried out. The results showed that antimicrobial efficacy of both QAS copolymer (PMT-5% and PMT-10%) coatings are as effective as that of QAS monomeric agent (DTPAC) coating against *S. aureus* and *E. coli* no matter coated on glass or zirconia and silica containing Lava restoratives. However, the antimicrobial result of QAS coatings against *C. albicans* was different from that of QAS solutions. DTPAC, the monomeric QAS agent was not effective against *C. albicans* upon coating although it is effective against *C. albicans* in solutions. More interestingly, the QAS copolymers are effective against *C. albicans* upon coating on glass surfaces although ineffective against *C. albicans* in solutions. Compared to QAS copolymer coatings on glass, the antimicrobial effect of QAS copolymer treated Lava restoratives was less effective against *C. albicans* although there was a significant reduction in fungus number compared to control.

Our results further confirmed previous observations that antimicrobial activity in solution is not directly indicative of their real properties upon attachment to a surface.[6,16] High surface concentration of antimicrobial groups (cationic quaternary ammonium groups) may make surface-attached polymers bactericidal to microbes resistant to these polymers in solution.[6] Besides, a higher hydrophobicity was obtained with the QAS copolymer coating, which may lead to higher resistance to bacterial adherence.[41] Furthermore, the QAS copolymer coatings are supposed to have a higher mobility compared to monomeric QAS agents because of their long flexible alkyl backbone chain pendent with cationic quaternary ammonium groups, which may have an impact on the antimicrobial activity as well.

After antimicrobial experiments, the substrates were rinsed and dried, then subjected to ATR–FTIR testing again to check the durability of QAS coatings after bacterial challenge. The characteristic peak of copolymers still remained even though the coatings were subjected to vigorous solution mixing and rinsing during the previous antimicrobial assays. It not only demonstrated strong adhesion of polymer coatings, but also the high stability of the polymer coatings (Fig. S2†). Ideally, the activity of these QAS coatings shall be permanent because their quaternary ammonium groups are not consumed during the biocidal process.

The QAS copolymers are expected to be able to be coated on a wide range of substrates and exhibit durable antimicrobial activities similar to other QAS monomeric compounds.[45] The coating on glass serves as a proof of concept. Glass has many applications, including packing, windows, optical lenses and medical devices. Since the QAS copolymers have negligible effects on the appearance of treated surfaces, it can maintain the transparency of glass and also protect the glass from possible bacterial contamination. Our study has demonstrated the low toxicity of the QAS copolymer and the superior antimicrobial efficacy of QAS copolymer coatings on the glass surfaces, which are expected to provide a long-lasting effect in inhibiting microbial infection. At the same time, we also noticed that the copolymers behaved a bit differently when coated on a different material, Lava restoratives, with a lower antimicrobial activity against *C. albicans* as compared to the coatings on glass. In future studies, the coating of our synthesized polymers may be tested on more substrates to explore the potential application in medical devices. The suggested substrates include metals such as titanium (orthopaedic and dental implants) and polymers such as polyurethane or silicon (in catheters). Furthermore, the adhesion strength of our polymers may be evaluated to further investigate the coating durability.

Conclusion

Novel antimicrobial QAS copolymers, namely, PMT-5% and PMT-10%, have been successfully synthesized from MAPTAC and TMSPMA monomers. The synthesis is not labor-intensive or costly, rendering their production potentially feasible on an industrial scale. Cytotoxicity assays with HDF cells, HaCaT keratinocytes and HEK293 cell lines indicated that the QAS copolymers are less toxic to human cells than a commercial QAS monomeric antimicrobial agent. Moreover, QAS copoly-

mers were capable of forming effective antimicrobial coatings against both bacteria (*S. aureus* and *E. coli*) and fungus (*C. albicans*) *via* a facile thermal-curing process. Abrasion resistant results showed that QAS copolymer coatings are more durable than a coating formed by DTPAC. The new QAS copolymers have a great potential to be used as a safer substitute for the current monomeric QAS coating agents.

Acknowledgements

The study was supported by a Singapore EDB-IPP grant (grant number: RL2012-035). The authors thank Siew Ping Yeo and Shuangyong Sun from 3M Innovation Singapore for the assistance with SEM imaging and thermal analysis, respectively.

References

1 R. Kenawy el, S. D. Worley and R. Broughton, *Biomacromolecules*, 2007, **8**, 1359–1384.
2 C. Blaszykowski, S. Sheikh and M. Thompson, *Biomater. Sci.*, 2015, **3**, 1335–1370.
3 A. Trampuz and A. F. Widmer, *Curr. Opin. Infect. Dis.*, 2006, **19**, 349–356.
4 P. Tenke, C. R. Riedl, G. L. Jones, G. J. Williams, D. Stickler and E. Nagy, *Int. J. Antimicrob. Agents*, 2004, **23**(Suppl 1), S67–S74.
5 J. Song, H. Kong and J. Jang, *Colloids Surf., B*, 2011, **82**, 651–656.
6 F. Siedenbiedel and J. C. Tiller, *Polymer*, 2012, **4**, 46–71.
7 R. Advincula, in *Surface-initiated polymerization I*, ed. R. Jordan, Springer Berlin Heidelberg, 2006, vol. 197, ch. 66, pp. 107–136.
8 P. T. Hammond, *AIChE J.*, 2011, **57**, 2928–2940.
9 J. Friedrich, *Plasma Processes Polym.*, 2011, **8**, 783–802.
10 A. Roosjen, W. Norde, H. Mei and H. Busscher, in *Characterization of Polymer Surfaces and Thin Films*, ed. K. Grundke, M. Stamm and H.-J. Adler, Springer Berlin Heidelberg, 2006, vol. 132, ch. 26, pp. 138–144.
11 G. Cheng, H. Xue, Z. Zhang, S. Chen and S. Jiang, *Angew. Chem., Int. Ed.*, 2008, **47**, 8831–8834.
12 H. A. Pearson, J. M. Andrie and M. W. Urban, *Biomater. Sci.*, 2014, **2**, 512–521.
13 M. Ramstedt, N. Cheng, O. Azzaroni, D. Mossialos, H. J. Mathieu and W. T. Huck, *Langmuir*, 2007, **23**, 3314–3321.
14 D. Alves and M. Olivia Pereira, *Biofouling*, 2014, **30**, 483–499.
15 M. Kazemzadeh-Narbat, B. F. Lai, C. Ding, J. N. Kizhakkedathu, R. E. Hancock and R. Wang, *Biomaterials*, 2013, **34**, 5969–5977.
16 G. Gao, D. Lange, K. Hilpert, J. Kindrachuk, Y. Zou, J. T. Cheng, M. Kazemzadeh-Narbat, K. Yu, R. Wang, S. K. Straus, D. E. Brooks, B. H. Chew, R. E. Hancock and J. N. Kizhakkedathu, *Biomaterials*, 2011, **32**, 3899–3909.
17 J.-Y. Wach, S. Bonazzi and K. Gademann, *Angew. Chem., Int. Ed.*, 2008, **47**, 7123–7126.
18 F. Zhang, Z. L. Shi, P. H. Chua, E. T. Kang and K. G. Neoh, *Ind. Eng. Chem. Res.*, 2007, **46**, 9077–9086.
19 N. Aumsuwan, S. Heinhorst and M. W. Urban, *Biomacromolecules*, 2007, **8**, 713–718.
20 N. Aumsuwan, M. S. McConnell and M. W. Urban, *Biomacromolecules*, 2009, **10**, 623–629.
21 F. Tang, L. Zhang, Z. Zhang, Z. Cheng and X. Zhu, *J. Macromol. Sci., Part A: Pure Appl. Chem.*, 2009, **46**, 989–996.
22 F. Costa, I. F. Carvalho, R. C. Montelaro, P. Gomes and M. C. Martins, *Acta Biomater.*, 2011, **7**, 1431–1440.
23 G. T. Qin, A. Lopez, C. Santos, A. M. McDermott and C. Z. Cai, *Biomater. Sci.*, 2015, **3**, 771–778.
24 W. M. Cho, B. P. Joshi, H. Cho and K. H. Lee, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 5772–5776.
25 M. Bagheri, M. Beyermann and M. Dathe, *Antimicrob. Agents Chemother.*, 2009, **53**, 1132–1141.
26 K. Hilpert, M. Elliott, H. Jenssen, J. Kindrachuk, C. D. Fjell, J. Korner, D. F. Winkler, L. L. Weaver, P. Henklein, A. S. Ulrich, S. H. Chiang, S. W. Farmer, N. Pante, R. Volkmer and R. E. Hancock, *Chem. Biol.*, 2009, **16**, 58–69.
27 S. A. Onaizi and S. S. Leong, *Biotechnol. Adv.*, 2011, **29**, 67–74.
28 J. C. Tiller, S. B. Lee, K. Lewis and A. M. Klibanov, *Biotechnol. Bioeng.*, 2002, **79**, 465–471.
29 S. B. Lee, R. R. Koepsel, S. W. Morley, K. Matyjaszewski, Y. Sun and A. J. Russell, *Biomacromolecules*, 2004, **5**, 877–882.
30 A. J. Isquith, E. A. Abbott and P. A. Walters, *Appl. Microbiol.*, 1972, **24**, 859–863.
31 L. Windler, M. Height and B. Nowack, *Environ. Int.*, 2013, **53**, 62–73.
32 USEPA, Reregistration eligibility decision for trimethoxysilyl quaternary ammonium chloride compounds, Report EPA 739-R-07-007, United States Environmental Protection Agency, Prevention, Pesticides and Toxic Substances, Washington, DC, 2007.
33 A. Ruiz Oropeza, U. Fischer Friis and J. Duus Johansen, *Contact Dermatitis*, 2011, **64**, 297–298.
34 M. Iwata, H. Tanizaki, H. Fujii, Y. Endo, A. Fujisawa, M. Tanioka, Y. Miyachi and K. Kabashima, *Acta Derm.-Venereol.*, 2015, **95**, 628–629.
35 S. Imazato, Y. Kinomoto, H. Tarumi, S. Ebisu and F. R. Tay, *Dent. Mater. J.*, 2003, **19**, 313–319.
36 S. Egger, R. P. Lehmann, M. J. Height, M. J. Loessner and M. Schuppler, *Appl. Environ. Microbiol.*, 2009, **75**, 2973–2976.
37 S. Chen, S. Chen, S. Jiang, Y. Mo, J. Luo, J. Tang and Z. Ge, *Colloids Surf., B*, 2011, **85**, 323–329.
38 G. L. French, *J. Antimicrob. Chemother.*, 2006, **58**, 1107–1117.
39 A. M. Carmona-Ribeiro and L. D. de Melo Carrasco, *Int. J. Mol. Sci.*, 2013, **14**, 9906–9946.
40 S. Q. Gong, L. N. Niu, L. K. Kemp, C. K. Yiu, H. Ryou, Y. P. Qi, J. D. Blizzard, S. Nikonov, M. G. Brackett, R. L. Messer, C. D. Wu, J. Mao, L. Bryan Brister,

F. A. Rueggeberg, D. D. Arola, D. H. Pashley and F. R. Tay, *Acta Biomater.*, 2012, **8**, 3270–3282.
41 J. Song, H. Kong and J. Jang, *Colloids Surf., B*, 2011, **82**, 651–656.
42 P. J. Eginton, H. Gibson, J. Holah, P. S. Handley and P. Gilbert, *Colloids Surf., B*, 1995, **5**, 153–159.
43 A. Hamza, V. A. Pham, T. Matsuura and J. P. Santerre, *J. Membr. Sci.*, 1997, **131**, 217–227.
44 C. I. Pereni, Q. Zhao, Y. Liu and E. Abel, *Colloids Surf., B*, 2006, **48**, 143–147.
45 A. J. Isquith, E. A. Abbott and P. A. Walters, *Appl. Microbiol.*, 1972, **24**, 859–863.