Preparation and Evaluation of an Antibacterial Dental Cement Containing Quaternary Ammonium Salts

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ABSTRACT: A novel PQAS-containing antibacterial glass-ionomer cement has been developed. The functional QAS and their constructed PQAS were synthesized, characterized, and formulated into the light-cured cements. Compressive strength (CS) and bacterial (*S. mutans* and lactobacillus) viability were used to evaluate the mechanical strength and antibacterial activity of the cements. Flexural (FS) and diametral tensile strengths (DTS) were tested as well. Fuji II LC cement was used as control. The specimens were conditioned in distilled water at 37°C for 24 h prior to testing. All the PQAS-containing cements showed a significant antibacterial activity, accompanying with an initial CS reduction. The effects of chain length, loading, and grafting ratio of the QAS were significant. Increasing chain length, loading, grafting ratio significantly enhanced antibacterial activity but reduced the initial CS of the formed cements. The antibacterial effect of the substitute chain lengths from free QAS seem more significant in water than those from their polymers (PQAS) after integrating to the cement. The experimental cement showed less CS reduction and higher antibacterial activity than Fuji II LC. The long-term aging study indicates that the cement might have a long-lasting antibacterial function with no PQAS leaching. Within the limitations of this study, it appears that the experimental cement is a clinically attractive dental restorative that can be potentially used for long-lasting restorations due to its high mechanical strength and long-lasting antibacterial function. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 2542–2551, 2011

Key words: PQAS; substitute chain length; antibacterial; *S. mutans* viability; glass-ionomer cement; CS; aging

INTRODUCTION

Long-lasting restoratives and restoration are clinically attractive because they can reduce patients' pain and expense as well as the number of their visits to dental offices.[1–4] In dentistry, both restorative materials and oral bacteria are believed to be responsible for restoration failure.[2] Secondary caries is found to be the main reason to the restoration failure of either dental composite resins or glass-ionomer cements (GICs).[1–4] Secondary caries that often occur at the interface between the restoration and the cavity preparation is primarily caused by demineralization of tooth structure due to invasion of plaque bacteria (acid-producing bacteria) such as *Streptococcus mutans* (*S. mutans*) and lactobacilli in the presence of fermentable carbohydrates.[4] To make long-lasting restorations, the materials should be made antibacterial. Among all the dental restoratives, GICs are found to be the most cariostatic and somehow antibacterial due to release of fluoride, which is believed to help reduce demineralization, enhance remineralization, and inhibit microbial growth.[5,6] However, annual clinical surveys found that secondary caries was still the main reason for GIC failure,[1–4] indicating that the fluoride release from GICs is not potent enough to inhibit bacterial growth or combat bacterial destruction. Although numerous efforts have been made on improving antibacterial activities of dental restoratives, most of them have been focused on release or slow-release of various incorporated low molecular weight (MW) antibacterial agents such as antibiotics, zinc ions, silver ions, iodine, and chlorhexidine (CHX).[6–10] Yet release or slow release can lead or has led to a reduction of mechanical properties of the restoratives over time, short-term effectiveness, and possible toxicity to surrounding tissues if the dose or release is not properly controlled.[6–10]

Polymers containing quaternary ammonium (QAS) or phosphonium salt (QPS) groups have been studied extensively as an important antimicrobial material and used for a variety of applications due to their potent antimicrobial activities.[11–15] These polymers are found to be capable of killing bacteria that are resistant to other types of cationic antibacterials.[16] The examples of polyQAS or PQAS used as

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antibacterials for dental restoratives include incorporation of a methacryloyloxydodecyl pyridinium bromide (MDPB) as an antibacterial monomer into composite resins,[13] use of methacryloxylethyl cetyl ammonium chloride (DMAE-CB) as a component for antibacterial bonding agents,[17,18] and incorporation of quaternary ammonium polyethylenimine (PEI) nanoparticles into composite resins.[19] All these studies found that PQAS did exhibit significant antibacterial activities. However, so far there have been no reports on using PQAS as an antibacterial agent for GICs.

The objective of this study was to synthesize a new poly(acrylic acid-*co*-itaconic acid) with pendent quaternary ammonium salt (PQAS) and explore the effects of this PQAS on the mechanical strength and antibacterial activity of commercial Fuji II LC and recently developed experimental high-strength cements.

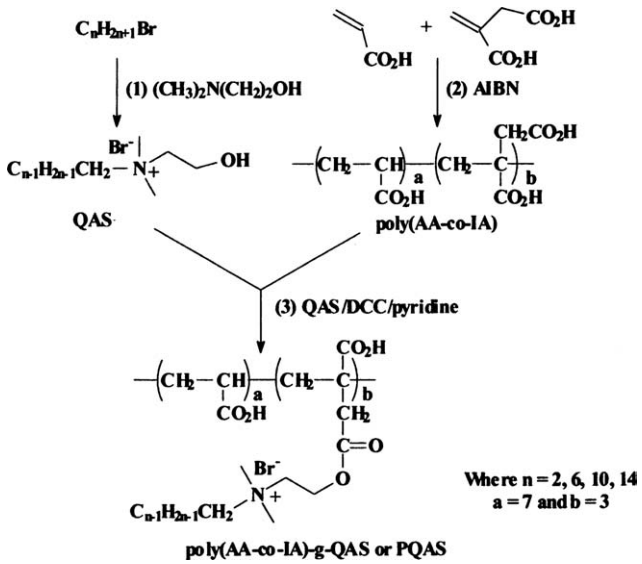

Figure 1 Synthesis scheme for QAS and PQAS: (a) synthesis of QAS; (b) synthesis of the 6-arm star-shape poly (AA-*co*-IA), followed by tethering QAS onto the polymer.

MATERIALS AND METHODS

Materials

Bromoethane, bromohexane, bromodecane, bromdodecane, bromotetradecane, bromohexadecane, 2-dimethylaminoethanol (DMAE), dipentaerythritol, 2-bromoisobutyryl bromide (BIBB), acrylic acid (AA), itaconic acid (IA), 2,2′-azobisisobutyronitrile (AIBN), triethylamine (TEA), copper (I) bromide (CuBr), *N*,*N*,*N*′,*N*′,*N*′-pentamethyldiethylenetriamine (PMDETA), *dl*-camphoroquinone (CQ), 2-(dimethylamino)ethyl methacrylate (DMAEMA), pyridine, *tert*-butyl acrylate (*t*-BA), glycidyl methacrylate (GM), hydrochloric acid (HCl, 37%), N,N′-dicyclohexylcarbodiimide (DCC), pyridine, diethyl ether, dioxane, *N*,*N*-dimethylformamide (DMF), methanol (MeOH), ethyl acetate (EA), hexane, and tetrahydrofuran (THF) were used as received from VWR International, Inc. (Bristol, CT) without further purifications. Light-cured glass-ionomer cement Fuji II LC and Fuji II LC glass powders were used as received from GC America Inc. (Alsip, IL).

Synthesis and characterization

Synthesis of the quaternary ammonium salt (QAS)

The hydroxyl group-containing quaternary ammonium salt (QAS) was synthesized following the procedures described elsewhere with a slight modification.[12] Briefly, to a flask containing DMAE (0.056 mol) in methanol, bromohexane (0.062 mol) was added. The reaction was run at room temperature overnight. After most of methanol was removed, the mixture was washed with hexane three times. The formed 2-dimethyl-2-hexyl-1-hydroxyethyl ammonium bromide or B6 was purified by dissolving in methanol and washing with hexane several times before drying in a vacuum oven. The synthesis scheme is shown in Figure 1.

Synthesis of the poly(acrylic acid-co-itaconic acid) with pendent QAS

The linear poly(acrylic acid-*co*-itaconic acid) or poly (AA-*co*-IA) was prepared following our published procedures.[20] Briefly, to a flask containing a solution of AA (0.08 mol) and IA (0.04 mol) in 40 mL THF, AIBN (0.5 mmol) in 10 mL THF was added. After the reaction was run under N_2 purging at 60°C for 18 h, the polymer was precipitated with ether, followed by drying in a vacuum oven. Then B6 was tethered onto the purified polymer.[21] Briefly, to a solution of poly(AA-*co*-IA) in DMF, B6 was added with DCC and pyridine. The reaction was run at room temperature overnight. After the insoluble dicyclohexyl urea was filtered off, the formed polymer with pendent QAS or PQAS was purified by precipitation from ether, washing with ether, and drying in a vacuum oven prior to use (see Fig. 1).

Synthesis of the GM-tethered star-shape poly(acrylic acid)

The GM-tethered 6-arm star-shape poly(acrylic acid) or poly(AA) was synthesized similarly as described in our previous publication.[22] Briefly, dipentaerythritol (0.06 mol) in 200 mL THF was used to react with BIBB (0.48 mol) in the presence of TEA (0.35 mol) to form the 6-arm initiator. *t*-BA (0.078 mol) in 10 mL dioxane was then polymerized with the 6-arm initiator (1% by mole) at 120°C in the presence of a CuBr (3%)-PMDETA (3%) catalyst complex via atom transfer radical polymerization. The resultant 6-arm poly(*t*-BA) was hydrolyzed with HCl and dialyzed

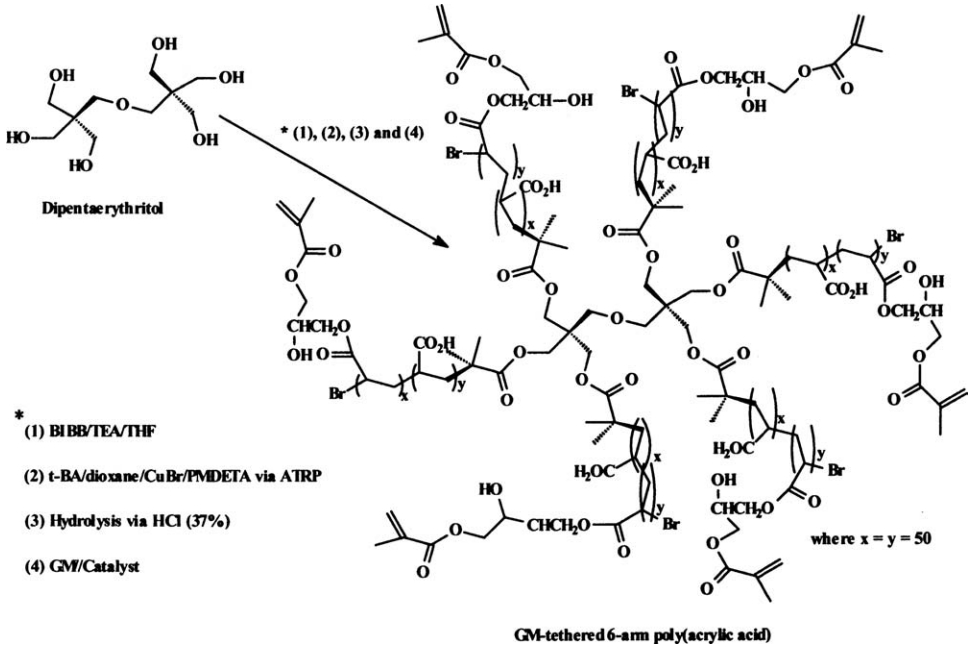

Figure 2 Schematic diagram for synthesis of the 6-arm star-shape poly(AA) tethered with polymerizable methacrylate groups.

against distilled water. The purified star-shape poly (AA) was obtained via freeze drying, followed by tethering with GM (50% by mole) in DMF in the presence of pyridine (1% by weight).[22] The GM-tethered star-shape poly(AA) was recovered by precipitation from diethyl ether, followed by drying in a vacuum oven at room temperature. The synthesis scheme for the 6-arm star-shape poly(AA) tethered with polymerizable methacrylate groups is shown in Figure 2.

Characterization

The chemical structures of the synthesized QAS and PQAS were characterized by Fourier transform-infrared (FT-IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. The proton NMR (^{1}HNMR) spectra were obtained on a 500 MHz Bruker NMR spectrometer (Bruker Avance II, Bruker BioSpin Corp., Billerica, MA) using deuterated dimethyl sulfoxide and chloroform as solvents and FT-IR spectra were obtained on a FT-IR spectrometer (Mattson Research Series FT/IR 1000, Madison, WI).

Evaluation

Sample preparation for mechanical strength tests

The experimental GIC (EXPGIC) cements were formulated with a two-component system (liquid and powder).[22] The liquid was formulated with the light-curable 6-arm star-shape poly(AA), water, 0.9% CQ (photo-initiator, by weight), and 1.8% DMAEMA (activator). The polymer/water (P/W) ratios (by weight) = 70 : 30. Fuji II LC glass powder was either used alone or mixed with the synthesized PQAS to formulate the cements, where the PQAS mixing ratio (by weight) was used at a predetermined ratio of the glass. The detailed formulations are shown in Table II. Fuji II LC (a two-component system with glass powder and polymer/resin/water liquid) was used as control and prepared per manufacturer's instruction where the powder/liquid (P/L) ratio = 3.2.

Specimens were fabricated at room temperature according to the published protocol.[22] Briefly, the cylindrical specimens were prepared in glass tubing with dimensions of 4 mm in diameter by 8 mm in length for compressive strength (CS), 4 mm in diameter by 2 mm in length for diametral tensile strength (DTS), and 4 mm in diameter by 2 mm in depth for antibacterial tests. The rectangular specimens were prepared in a split Teflon mold with dimensions of 3 mm in width by 3 mm in thickness by 25 mm in length for flexural strength (FS) test. All the specimens were exposed to blue light (EXAKT 520 Blue Light Polymerization Unit, EXAKT Technologies, Inc., Oklahoma City, OK) for 2 min, followed by conditioning in 100% humidity for 15 min, removed from the mold, and conditioned in distilled water at 37°C for 24 h unless specified, prior to testing.

Strength measurements

CS, DTS, and FS tests were performed on a screw-driven mechanical tester (QTest QT/10, MTS Systems Corp., Eden Prairie, MN), with a crosshead speed of 1 mm/min. The FS test was performed in three-point bending with a span of 20 mm between

supports. Six to eight specimens were tested to obtain a mean value for each material or formulation in each test. CS was calculated using an equation of $CS = P/\pi r^2$, where P = the load at fracture and r = the radius of the cylinder. DTS was determined from the relationship $DTS = 2P/\pi dt$, where P = the load at fracture, d = the diameter of the cylinder, and t = the thickness of the cylinder. FS was obtained using the expression $FS = 3Pl/2bd^2$, where P = the load at fracture, l = the distance between the two supports, b = the breadth of the specimen, and d = the depth of the specimen.

MIC test for the synthesized QAS

The minimal inhibitory concentration or MIC of the synthesized QAS was determined following the published protocol with a slight modification.[23] Briefly, colonies of *S. mutans* (UA159) were suspended in 5 mL of Tryptic soy Broth (TSB) prior to MIC testing. Two-fold serial dilutions of the synthesized QAS were prepared in TSB, followed by being placed into 96-well flat-bottom microtiter plates with a volume of 250 μL per well. The final concentration of the QAS ranged from 1.563 to 2×10^4 μg/mL. The microtiter plate was then inoculated with *S. mutans* suspension (cell concentration = 5×10^5 CFU/mL) and incubated at 37°C for 48 h prior to MIC testing. The absorbance was measured at 595 nm via a microplate reader (SpectraMax 190, Molecular Devices, CA) to assess the cell growth. Chloehexidine (CHX) and dimethylsulfoxide were used as positive and negative controls, respectively. Triple replica was used to obtain a mean value for each QAS.

Antibacterial test for the formed cements

The antibacterial test was conducted following the published procedures.[24] Both *S. mutans* and lactobacillus were used for evaluation of antibacterial activity of the studied cements. Briefly, colonies of *S. mutans* or lactobacillus were suspended in 5 mL of tryptic soy broth (TSB), supplemented with 1% sucrose, to make a suspension with 10^8 CFU/mL of bacteria, after 24 h incubation. Specimens pretreated with ethanol (10 s) were incubated with bacteria in TSB at 37°C for 48 h under 5% CO_2. After equal volumes of the red and the green dyes (LIVE/DEAD BacLight bacterial viability kit L7007, Molecular Probes, Inc., Eugene, OR, USA) were combined in a microfuge tube and mixed thoroughly for 1 min, 3 μL of the dye mixture was added to 1 mL of the bacteria suspension, mixed by vortexing for 10 s, sonicating for 10 s as well as vortexing for another 10 s, and kept in dark for about 15 min, prior to analysis. Then 20 μL of the stained bacterial suspension was analyzed using a fluorescent microscope (Nikon Microphot-FXA, Melville, NY, USA). Triple replica was used to obtain a mean value for each material.

Statistical analysis

One-way analysis of variance (ANOVA) with the post hoc Tukey-Kramer multiple-range test was used to determine significant differences of mechanical strength and antibacterial tests among the materials in each group. A level of $\alpha = 0.05$ was used for statistical significance.

RESULTS

Characterization

The characteristic chemical shifts (ppm) from the ^{1}HNMR spectra (see Fig. 3) for bromohexane, DMEA, B6, poly(AA-*co*-IA), and poly(AA-*co*-IA) with pendent B6 or namely PQAS are shown below: (a) bromohexane: 3.51 (—CH_2Br), 1.80 (—CH_2CH_2Br), 1.38 (—CH_2—, all), and 0.89 (—CH_3); (b) DMEA: 4.40 (—OH), 3.42 (—CH_2OH), 2.30 (—CH_2N—), and 2.10 (H_3CN—); (c) B6: 5.20 (—OH), 3.82 (—CH_2OH), 3.45 (—CH_2N$(CH_3)_2$), 3.15 (H_3CN—), 1.65 (—CH_2CH_2N$(CH_3)_2$), 1.25 (—CH_2— all), and 0.89 (—CH_3); (d) poly(AA-*co*-IA): 12.2 (—COOH), 3.45 (—CH(COOH) —), and 1.2–2.5 (—CH_2—, all); (e) PQAS: 3.80 (—CH_2(COOH) —), 3.30–3.45 (—CH_2N—), 3.10 (H_3CN—), 1.65 (—CH_2CH_2N$(CH_3)_2$), 1.25 (—CH_2— all), and 0.89 (—CH_3). The appearance of all the new peaks in the spectrum for PQAS confirmed the successful attachment of B6 onto the poly(AA-*co*-IA).

The characteristic peaks (cm^{-1}) from the FT-IR spectra (see Fig. 4) for bromohexane, DMEA, B6, poly(AA-*co*-IA), and PQAS are listed below: (a) bromohexane: 2920 (C—H stretching on —CH_2—), 2851 (C—H stretching on —CH_3), 1464, 1375, and 1250 (C—H deformation on —CH_2—), and 721 as well as 647 (C—Br deformation); (b) DMEA: 3399 (O—H stretching), 2944 (C—H stretching on —CH_2—), 2861 (C—H stretching on —CH_3), 2820, and 2779 (C—H stretching on —N$(CH_3)_2$), 1459, 1364, and 1268 (C—H deformation on —CH_2—), 1090 (O—H deformation), and 1040 as well as 776 (C—N deformation); (c) B6: 3600–3200 (=N^+= stretching), 2917 (C—H stretching on —CH_2—), 2850 (C—H stretching on —CH_3), 1470 (C—H deformation on —CH_2—), and 1090 as well as 730 (O—H deformation); (d) poly(AA-*co*-IA): 3800–2400 (O—H stretching on —COOH), 1716 (—C=O stretching), and 1458 (C—H deformation on —CH_2—); (e) PQAS: 3353 (=N^+=stretching), 3800–2400 (O—H stretching on —COOH), 2923 (C—H stretching on —CH_2—), 2853 (C—H stretching on —CH_3), 1732 (—C=O stretching), 1466 (C—H deformation on —CH_2—) and 776 (C—N deformation).

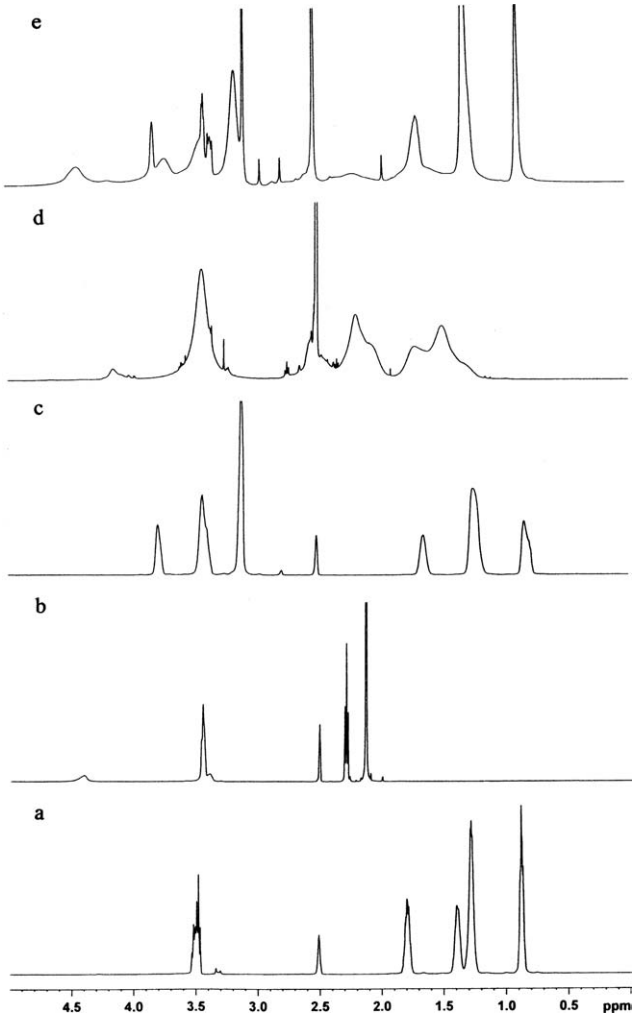

Figure 3 [1]HNMR spectra: (a) bromohexane; (b) DMEA; (c) B6; (d) poly(AA-*co*-IA), and (e) PQAS.

The significant peaks at 3600–3200 for $=N^{+}=$group, 2923 and 2853 for $-CH_2-$ group and 1736 for carbonyl group confirmed the formation of PQAS.

Evaluation

Table I shows the code, description, and MIC of the synthesized QAS. The MIC values ranged from 1.563 to 20,000 μg/mL for B16 to B2.

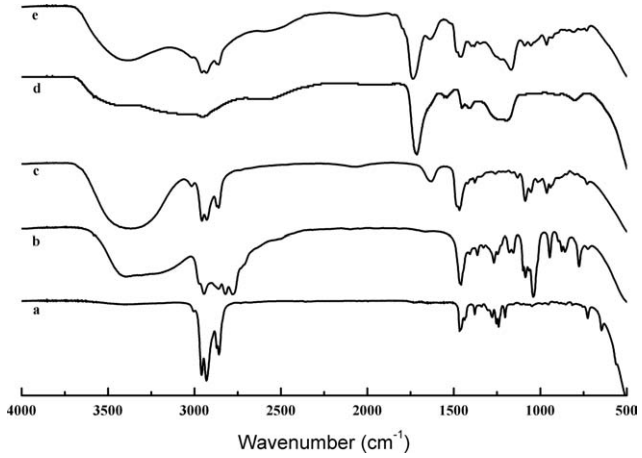

Figure 4 FT-IR spectra: (a) bromohexane; (b) DMEA; (c) B6; (d) poly(AA-*co*-IA), and (e) PQAS.

Table II shows the effect of the substitute chain length of the synthesized QAS on CS of both Fuji II LC and EXPGIC cements, and *S. mutans* as well as lactobacillus viabilities. EXPGIC stands for experimental GIC whereas Fuji II LC represents commercial GIC. EXPGIC (B2), EXPGIC (B6), EXPGIC (B10), and EXPGIC (B14) stand for the PQAS polymers tethered with different chain length of QAS bromide in EXPGIC and so do Fuji II LC (see details in Tables I and II). Fuji II LC and EXPGIC without PQAS addition were used as controls. The mean CS value (MPa) was in the decreasing order of EXPGIC > Fuji II LC > EXPGIC (B2) > EXPGIC (B6) > EXPGIC (B10) > EXPGIC (B14) > Fuji II LC (B2) > Fuji II LC (B6) > Fuji II LC (B10) > Fuji II LC (B14). The PQAS addition significantly decreased the CS values of the cements, with a reduction of 50–60% for Fuji II LC and 37–52% for EXPGIC. Increasing the substitute chain length on the QAS decreased the CS values of both cements but the decreasing rate was not dramatic. The mean *S. mutans* viability was in the decreasing order of Fuji II LC > EXPGIC > Fuji II LC (B2) > Fuji II LC (B6) > Fuji II LC (B10) > EXPGIC (B2) > Fuji II LC (B14) > EXPGIC (B6) > EXPGIC (B10) > EXPGIC (B14). The PQAS addition significantly decreased the *S. mutans* viability, with a

TABLE I
Codes, Description, MIC Values of the Synthesized QAS

Code	QAS[a]	Chain length	MIC (ig/ml)[b]
B2	2-Dimethyl-2-ethyl-1-hydroxyethylammonium bromide	2 C	20,000
B6	2-Dimethyl-2-hexyl-1-hydroxyethylammonium bromide	6 C	1,000
B10	2-Dimethyl-2-decyl-1-hydroxyethylammonium bromide	10 C	200
B12	2-Dimethyl-2-dodecyl-1-hydroxyethylammonium bromide	12 C	25
B14	2-Dimethyl-2-tetradecyl-1-hydroxyethylammonium bromide	14 C	1.563
B16	2-Dimethyl-2-hexadecyl-1-hydroxyethylammonium bromide	16 C	1.563

[a] All the QAS were freshly synthesized and water-soluble.
[b] MIC values were measured as described in the text.

TABLE II
Effect of the Chain Length of the Synthesized QAS on CS and Antibacterial Activity of the Cements

Material[1]	Chain length[2]	CS (MPa)	*S. mutans* viability (%)	Lactobacillus viability (%)
FIILC	–	237.9 (4.5)[3]	77.9 (2.7)[d]	76.3 (2.1)[i]
FIILC(B2)	2	117.8 (1.3)[a]	48.1 (2.8)[e]	51. 2 (1.3)
FIILC(B6)	6	112.3 (2.1)[a]	43.7 (1.3)[e,f]	37.7 (2.4)[j]
FIILC(B10)	10	96.1 (2.8)[a]	37.7 (1.4)[f]	33.2 (0.3)[j,k]
FIILC(B14)	14	95.9 (3.7)[a]	34.4 (1.3)[f,g]	28.7 (0.7)[k]
EXP	–	325.3 (4.2)	76.2 (3.5)[d]	75.1 (1.7)[i]
EXP(B2)	2	204.1 (3.8)[b]	36.4 (1.5)[f]	36.8 (2.4)[j]
(2.4)EXP(B6)	6	198.7 (4.7)[b]	28.3 (0.5)[g]	22.1 (1.5)[l]
EXP(B10)	10	163.8 (1.2)[c]	23.4 (1.6)[h]	20.7 (0.5)[l]
EXP(B14)	14	157.5 (1.2)[c]	20.9 (0.9)[h]	19.7 (0.6)[l]

[1] FIILC = Fuji II LC; EXP = EXPGIC; PQAS was mixed with Fuji II LC glass fillers, where PQAS = 7% (by weight) of glass fillers and QAS grafting ratio = 50%; For Fuji II LC cements, P/L = 3.2; For experimental cements, M_W of the 6-arm poly(AA) = 17,530 Daltons; GM-grafting ratio = 50%; P/L ratio = 2.7; P/W ratio = 70 : 30; B2-B14 stands for the QAS bromide with the substitute chain length from carbon 2 to 14.
[2] Chain length = the substitute chain length on QAS.
[3] Entries are mean values with standard deviations in parentheses and the mean values with the same superscript letter were not significantly different ($p > 0.05$). Specimens were conditioned in distilled water at 37°C for 24 h, followed by direct testing for CS and incubating with bacteria for 48 h for antibacterial testing.

reduction of 38–56% for Fuji II LC and 52–73% for EXPGIC. The mean lactobacillus viability was in the decreasing order of Fuji II LC > EXPGIC > Fuji II LC (B2) > Fuji II LC (B6) > EXPGIC (B2) > Fuji II LC (B10) > Fuji II LC (B14) > EXPGIC (B6) > EXPGIC (B10) > EXPGIC (B14). Increasing the substitute chain length on the QAS decreased both *S. mutans* and lactobacillus viabilities but the antibacterial activity against lactobacillus was higher than that against *S. mutans*. Furthermore, the decreasing rate was not as dramatic as that for the MIC values for the QAS in water (see Table I). Moreover, the PQAS in EXPGIC showed a higher antibacterial activity to both bacteria than those in Fuji II LC.

Figure 5 shows the effect of the PQAS loading on CS and antibacterial activity of EXPGIC. Increasing the PQAS loading decreased CS and bacterial viability. With 1–20% PQAS loading, EXPGIC lost 18–80% of its original CS but killed 17–83% of *S. mutans* and 23–88% of lactobacillus correspondingly. The more the PQAS added, the lower the CS and bacterial viability. Figure 6 shows the effect of the QAS-grafting

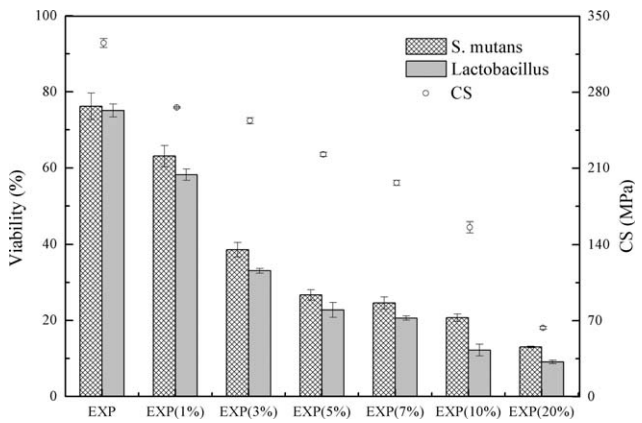

Figure 5 Effect of the PQAS content on CS and antibacterial activity of EXPGIC: the formulations were the same as those described in Table II, except for PQAS content change and QAS = B6. Specimens were conditioned in distilled water at 37°C for 24 h, followed by direct testing for CS and incubating with bacteria for 48 h for antibacterial testing.

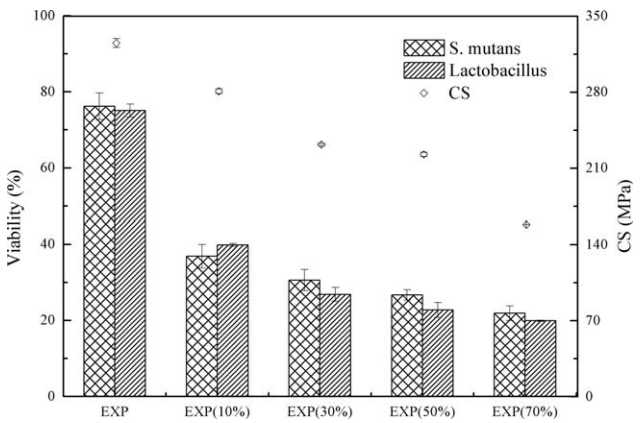

Figure 6 Effect of the QAS-grafting ratio on poly(AA-*co*-IA) on CS and antibacterial activity of EXPGIC: the formulations were the same as those described in Figure 5, except for PQAS content = 5%. Specimens were conditioned in distilled water at 37°C for 24 h, followed by direct testing for CS and incubating with bacteria for 48 h for antibacterial testing.

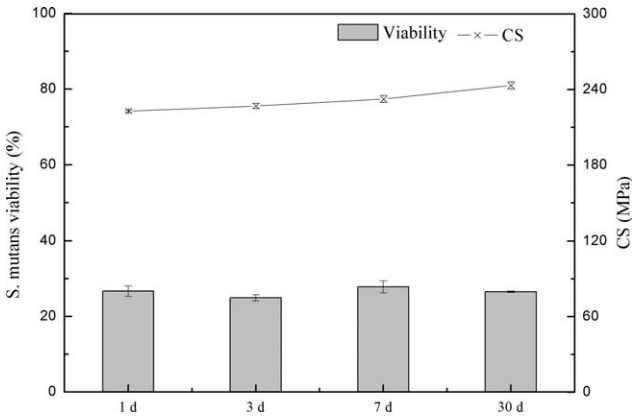

Figure 7 Effect of aging on both CS and *S. mutans* viability after culturing with experimental cements with and without PQAS addition: The formulation was the same as those described in Figure 6, except for QAS grafting ratio = 50%. The specimens were conditioned in distilled water at 37°C for 1 day, 3 days, 1 week, and 1 month, followed by direct testing for CS and incubating with *S. mutans* for 48 h for antibacterial testing.

ratio on poly(AA-*co*-IA) on CS and antibacterial activity of EXPGIC. Like the trend shown in Figure 5, increasing the QAS-grafting ratio decreased CS and bacterial viability. With increasing the QAS-grafting ratio from 10 to 70%, EXPGIC lost 13–51% of its original CS but killed 52–71% of *S. mutans* and 47–74% of lactobacillus correspondingly. Figure 7 shows the effect of the cement aging on CS and *S. mutans* viability. After 30-day aging in water, EXPGIC (B6) showed a slight increase in CS (statistically no difference) but no significant changes in the *S. mutans* viability. Table III shows the property comparison among Fuji II LC, EXPGIC, and EXPGIC (B6) with 5% PQAS loading and 50% QAS-grafting. For CS and modulus (M), the mean strength values were in the decreasing order of EXPGIC > Fuji II LC > EXPGIC-PQAS. For DTS and FS, EXPGIC > EXPGIC-PQAS > Fuji II LC. For both *S. mutans* and lactobacillus viabilities, Fuji II LC > EXPGIC > EXPGIC-PQAS.

DISCUSSION

Currently there is a growing interest in preventing or reducing biofilm formation in many biomedical areas. In preventive restorative dentistry, secondary caries is a critical issue and prevention of secondary caries plays a key role in long-lasting restorations.[1–4] Secondary caries is found to be the main reason to the restoration failure of either composite resins or glass-ionomer cements (GICs).[1–4] Secondary caries that often occurs at the interface between the restoration and the cavity preparation is mainly caused by demineralization of tooth structure due to invasion of plaque bacteria (acid-producing bacteria) such as *S. mutans* and lactobacilli in the presence of fermentable carbohydrates.[4] *S. mutans* are regarded as a primary invader to tooth cavities whereas lactobacilli together with *S. mutans* are considered as secondary invaders after primary invasion occurs.[25] However, both bacteria are believed to be the main oral bacteria which are responsible for secondary caries.[4,25] Therefore, preventing these two bacteria from invasion to natural tooth is the key to long-lasting dental restorations when the microleakage or materials failure occurs at the interface. PQAS represents a new trend of antimicrobial agents in biomedical applications.[11,14] PQAS can be incorporated in many ways, including mixing with fillers, copolymerizing with other monomers, and grafting onto the polymer skeletons.[11–15] The advantage of using QAS is that they can kill the microorganisms by simple contact. The mechanism of QAS to kill bacteria is believed to disrupt the surface membrane of bacteria by changing membrane permeability or surface electrostatic balance.[12,19] Unlike other leachable antibacterial agents such as silver ions, antibiotics, CHX, and low MW QAS, PQAS are not leachable due to their high

TABLE III
Comparison of Properties of Fuji II LC, EXPGIC, and PQAS-Containing EXPGIC[1]

Material	CS (MPa)	M[2] (GPa)	DTS[3] (MPa)	FS (MPa)	Viability[4] (%)	Viability[5] (%)
Fuji II LC	237.9 (4.5)[a,6]	6.91 (0.42)	43.4 (4.5)[b]	52.8 (1.9)[c]	77.9 (2.7)[d]	76.3 (2.1)[d]
EXPGIC	325.3 (4.2)	7.74 (0.04)	58.8 (0.2)	88.3 (2.5)	76.2 (3.5)[d]	75.1 (1.7)[d]
EXPGIC-PQAS	222.8 (1.9)[a]	5.62 (0.08)	49.8 (1.2)[b]	59.9 (2.3)[c]	26.7 (0.9)[e]	22.2 (0.7)[e]

[1] For PQAS-containing EXPGIC, M_W of the 6-arm poly(AA) = 17,530 Daltons, GM-grafting ratio = 50%, P/L ratio = 2.7, PQAS content = 5%, and QAS grafting ratio = 50%.
[2] M = compressive modulus.
[3] DTS = diametral tensile strength.
[4] Viability for *S. mutans*.
[5] Viability for lactobacillus.
[6] Entries are mean values with standard deviations in parentheses and the mean values with the same superscript letter in each category were not significantly different ($p > 0.05$). Specimens were conditioned in distilled water at 37°C for 24 h, followed by direct testing for CS and incubating with bacteria for 48 h for antibacterial testing.

MW.[15] In this regard, we purposely synthesized the new PQAS, incorporated it into our experimental high-strength cements, and evaluated the CS and antibacterial activity of the formed cements.

It has been noticed that chain length on QAS has a significant effect on its antibacterial activity.[12,15] Generally speaking, there are four main processes for PQAS to kill bacteria and they are (1) adsorption onto the negatively charged bacterial cell surface; (2) penetrating through the cell wall; (3) binding to the cytoplasmic membrane; and (4) disrupting the cytoplasmic membrane.[15] It has also been found that both positive charge density and substitute chain length are the key to the biocidal ability, because the high positive charge density may enhance the driving force and the long substitute chain may strongly interact with the cytoplasmic membranes.[15] From Table I, it is apparent that increasing the substitute chain length significantly increased the biocidal activity of the synthesized QAS. The QAS with 16-carbon substitute chain (B16) was the highest in MIC whereas the one with 2-carbon chain (B2) was the lowest. In fact, the trend for the biocidal activity of the QAS in this study was similar to those described elsewhere,[12,15] i.e., the longer the substitute chain, the higher the biocidal activity.

From the results in Table II, with PQAS addition both Fuji II LC and EXPGIC cements showed a decrease in CS and bacterial viabilities. Fuji II LC cements lost more CS (50–60% of its original 237 MPa) than EXPGIC did (37–52% of 325 MPa). The loss of CS can be attributed to the incorporated QAS because both charge and hydrophobic chain on the QAS did not contribute any strength enhancement to the cements. Regarding both *S. mutans* and lactobacillus viabilities, we found that both Fuji II LC and EXPGIC cements without PQAS addition killed about 20% *S. mutans* and lactobacilli, which can be attributed to the release of fluoride. It is known that GICs have inhibitory effects on bacteria due to its fluoride release.[6] With PQAS addition, both Fuji II LC and EXPGIC increased their antibacterial activity significantly. The longer the substitute chain, the higher the antibacterial activity. Moreover, EXPGIC showed an even stronger antibacterial activity than the corresponding Fuji II LC with increasing the chain length. The possible reason may be explained below. Since the synthesized PQAS is composed of 50% carboxylic acid and 50% QAS and both components are very hydrophilic, they are likely to have interactions with other hydrophilic components from the cement in the presence of water. Let's take a look at the compositions in polymer liquids from both cements. EXPGIC contains only hydrophilic GM-tethered poly(AA) (70%) and water (30%), whereas Fuji II LC contains a substantial amount (approximately 25–35%) of partially hydrophilic 2-hydroxyethyl methacrylate (HEMA) and highly hydrophobic dimethacrylaye/oligomethacrylte except for the linear poly(AA) (20–30%) and water (20–30%).[26] Therefore, the highly hydrophilic components in EXPGIC may help the PQAS chains better extend on the surface of the cements but the highly hydrophobic dimethacrylaye/oligomethacrylte and partially hydrophobic HEMA in Fuji II LC may restrict or interfere with the extension of the PQAS chains on the surface. Theoretically the more the QAS exposed the higher the antibacterial activity anticipated. The results imply that to reach the same or similar antibacterial results less PQAS might be required for EXPGIC than for Fuji II LC. The results also show that lactobacillus was more vulnerable to the synthesized PQAS than *S. mutans*, although PQAS killed both bacteria dramatically. We also noticed that EXPGIC (B6) showed a higher CS than either EXPGIC (B10) or EXPGIC (B14) but a similar antibacterial activity to both, meaning that B6 may be the optimal QAS for this GIC system based on CS and antibacterial activity.

The effect of the QAS (B6) loading on CS and antibacterial activity of EXPGIC is shown in Figure 5. Apparently, the more the synthesized PQAS was added the lower the CS values and the higher the antibacterial activity. To keep the CS value above 200 MPa and bacterial viabilities below 30%, EXPGIC (B6) with 5% PQAS loading seemed the best formulation. Therefore, we chose it to examine the effect of the QAS-grafting ratio on poly(AA-*co*-IA) on both CS and antibacterial activity. The result in Figure 6 shows that the higher the QAS-grafting ratio, the lower the CS, and the higher the antibacterial activity. However, both EXPGIC (B6) cements with 30 and 50% QAS-grafting ratios showed a CS value close to or above 220 MPa and bacterial viabilities close to or below 30%. Which one would be the optimal or appropriate formulation depends on what level of antibacterial activity of the cement we would anticipate.

As stated in introduction, most antibacterial dental materials rely on the release of chemicals or antibacterial agents including antibiotics, silver ions, zinc ions, etc.[6–10] However, release or slow release can lead or has led to a reduction of mechanical properties of the restoratives over time, short-term effectiveness, and possible toxicity to surrounding tissues if the dose or release is not properly controlled.[6–10] Our hypothesis was to develop an antibacterial glass-ionomer cement without being leachable; otherwise, both strength and antibacterial function would decrease with time or aging. To confirm if the incorporated PQAS was not leachable, we examined both CS and antibacterial function of EXPGIC (containing 5% PQAS) after aging in water for 1 day, 3 days, 1 week, and 1 month. The result in Figure 7 shows that there was a slight increase in CS but no

change or no reduction in antibacterial activity after one month of aging, indicating that there might be no PQAS leaching from the cement. The reason can be attributed to the fact that the PQAS synthesized in this study is the polyacid-containing polymer. It is known that the carboxylic acid group is the key to GIC setting and salt-bridge formation. The PQAS polymer incorporated not only provided QAS for antibacterial activity but also supplied carboxyl groups for salt-bridge formation. The latter helped the PQAS polymer to firmly attach to the glass fillers. Therefore, we may have developed a GIC with a long-lasting antibacterial function.

We also compared CS, compressive modulus (M), diametral tensile strength (DTS), flexural strength (FS), and antibacterial activity to both *S. mutans* and lactobacilli of the PQAS-containing EXPGIC with those of both EXPGIC and Fuji II LC without any PQAS addition. The PQAS-containing EXPGIC was 31% in CS, 27% in modulus, 15% in DTS, and 32% in FS lower than EXPGIC without PQAS addition and 6% in CS and 18% in modulus lower but 15% in DTS and 13% in FS higher than Fuji II LC. Furthermore, the PQAS-containing EXPGIC was much higher (65–66% higher) in antibacterial activity than both EXPGIC and Fuji II LC.

It has been noticed that most quaternary ammonium compounds show cytotoxicity to human cells more or less.[27,28] Although in this study we used the PQAS polymers instead of low MW QAS and the cured cements might not leach the PQAS, the PQAS release study is necessary to prove whether the incorporated PQAS is leachable or not, which will be part of our future work. The *in vitro* and *in vivo* biocompatibility or cytotoxicity of the formed cements to pulp and gingival cells will also need to be tested. It is known that wear is one of the major weaknesses for GICs.[5] Although the EXPGIC system used for this study demonstrated significantly high mechanical strengths and wear-resistance,[22,29] the new PQAS-containing EXPGIC system showed a significant decrease in CS, DTS, and FS as compared to EXPGIC but similar to Fuji II LC without PQAS addition. It is worthwhile to evaluate the wear-resistance of the PQAS-containing EXPGIC, because wear is a better indicator of clinical longevity than CS. Furthermore, if the PQAS-containing EXPGIC was used for simply cementing not as a filling restorative, we need to test how effective the very thin layer of the cement between the restorative and dental hard tissue would be on antibacterial activity. Therefore, more work needs to be done on this new antibacterial GIC in the near future.

CONCLUSIONS

We have developed a novel PQAS-containing antibacterial glass-ionomer cement. All the PQAS-containing cements showed a significant antibacterial activity, accompanying with an initial CS reduction. The effects of chain length, loading and grafting ratio of the QAS were significant. Increasing chain length, loading, and grafting ratio significantly enhanced antibacterial activity but reduced the initial CS of the formed cements. The antibacterial effect of the substitute chain lengths from free QAS seem more significant in water than those from their polymers (PQAS) after integrating to the cement. The experimental cement showed less CS reduction and higher antibacterial activity than Fuji II LC. The long-term aging study indicates that the cements might have a long-lasting antibacterial function with no PQAS leaching. Within the limitations of this study, it appears that the experimental cement is a clinically attractive dental restorative that can be potentially used for long-lasting restorations due to its high mechanical strength and long-lasting antibacterial function. Future studies will include testing the biocompatibility or cytotoxicity of this PQAS-containing GIC on pulp and gingival cells, studying the potential PQAS release of the cement and evaluating the wear-resistance of the cement.

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