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By Xia Guo

Entitled Preparation and Evaluation of Antibacterial Dental Glass-ionomer Cements

For the degree of Master of Science in Biomedical Engineering

Is approved by the final examining committee:

Dong Xie
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PREPARATION AND EVALUATION OF ANTIBACTERIAL DENTAL GLASS-IONOMER CEMENTS

A Thesis
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of
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by
Xia Guo

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ABSTRACT

Guo, Xia. M.S.B.M.E., Purdue University, December 2010. Preparation and Evaluation of Antibacterial Dental Glass-ionomer Cements. Major Professor: Dong Xie.

The functional quaternary ammonium salts (QAS) and their constructed polyQAS or PQAS were synthesized, characterized and formulated into a novel antibacterial glass-ionomer cement. Compressive strength (CS) and Streptococcus mutans (S. mutans) viability were used to evaluate the mechanical strength and antibacterial activity of the cements. Fuji II LC cement was used as control. The specimens were conditioned in distilled water at 37 ^{o}C for 24 h prior to testing. The effects of the substitute chain length, loading as well as grafting ratio of the QAS and aging on CS and S. mutans viability were investigated.

Chapter 2 describes how we studied and evaluated the formulated antibacterial glass-ionomer cement by incorporating QAS chloride-containing polymer into the formulation. The results show that with PQAS addition, the studied cements showed a reduction in CS with 25-95% for Fuji II LC and 13-78% for the experimental cement and a reduction in S. mutans viability with 40-79% for Fuji II LC and 40-91% for the experimental cement. The experimental cement showed less CS reduction and higher antibacterial activity as compared to Fuji II LC. The long-term aging study indicates that the cements are permanently antibacterial with no PQAS leaching.

Chapter 3 describes how we studied and evaluated the formulated antibacterial cements by changing chain length, type of halide, loading, grafting ratio and aging time. The results show that the effects of the chain length, loading and grafting ratio of the

QAS were significant. Increasing chain length, loading, grafting ratio significantly enhanced antibacterial activity but reduced CS. The experimental cement showed less CS reduction and higher antibacterial activity as compared to Fuji II LC. The long-term aging study indicates that the cements are permanently antibacterial with no PQAS leaching. There was no significant difference between QAS bromide and QAS chloride, suggesting that we can use QAS bromide directly without converting bromide to chloride.

In summary, we have developed a novel PQAS-containing antibacterial glass-ionomer cement. The cement has demonstrated significant antibacterial activities. Our experimental cement is a promising system because the reduced strength of the cement with addition of PQAS is still above those demonstrated by original commercial cement Fuji II LC without any PQAS addition. 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 permanent antibacterial function.

1. INTRODUCTION

1.1 Background

It is known that both restorative materials and oral bacteria are mainly responsible for the restoration failure. Secondary caries is found to be the main reason to the restoration failure of either composite resins or glass-ionomer cements (GICs). 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 *Streptococcus mutans (S. mutans)* in the presence of fermentable carbohydrates. 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. However, annual clinical surveys found that secondary caries was still the main reason for GIC failure, 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 antibacterial agents such as antibiotics, zinc ions, silver ions, iodine and chlorhexidine. 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.

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. These polymers are found to be capable of killing bacteria that are resistant to other types of cationic

antibacterials. The examples of polyQAS or PQAS used as antibacterials for dental restoratives include incorporation of a methacryloyloxydodecyl pyridinium bromide as an antibacterial monomer into composite resins and incorporation of quaternary ammonium polyethylenimine nanoparticles into composite resins. 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.

1.2. Hypothesis and Objectives

It is our hypothesis that incorporating PQAS into current or experimental glass-ionomer cement would provide a novel route for formulation of a novel antibacterial glass-ionomer cement for improved dental restoratives.

The objectives of the study in this thesis were: (1) to synthesize and characterize QAS and PQAS; (2) to formulate the GIC with the synthesized PQAS; (3) to evaluate the mechanical strengths of the formed cements; and (4) to evaluate the antibacterial activity of the formulated cements. Commercial Fuji II LC and recently developed experimental high-strength cements were used as controls.

Chapter 2 mainly describes the synthesis, characterization, formulation and evaluation of the cements composed of QAS chloride-containing polymers. Chapter 3 mainly describes the synthesis, characterization, formulation and evaluation of the cements composed of QAS bromide-containing polymers. The effects of chain length, type of halide, loading, grafting ratio and aging time on compressive strength and S. mutans viability were also investigated.

2. A PQAS-CONTAINING GLASS-IONOMER CEMENT FOR IMPROVED ANTIBACTERIAL FUNCTION

2.1 Abstract

The novel non-leachable poly(quaternary ammonium salt) (PQAS)-containing antibacterial glass-ionomer cement has been developed. Compressive strength (CS) and S. mutans viability were used as tools for strength and antibacterial activity evaluations, respectively. All the specimens were conditioned in distilled water at 37 ^{o}C prior to testing. Commercial glass-ionomer cement Fuji II LC was used as control. With PQAS addition, the studied cements showed a reduction in CS with 25-95% for Fuji II LC and 13-78% for the experimental cement and a reduction in S. mutans viability with 40-79% for Fuji II LC and 40-91% for the experimental cement. The experimental cement showed less CS reduction and higher antibacterial activity as compared to Fuji II LC. The long-term aging study indicates that the cements are permanently antibacterial with no PQAS leaching. 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 permanent antibacterial function.

2.2 Introduction

In dental clinics, 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 *Streptococcus mutans* (*S. mutans*) in the presence of fermentable carbohydrates [4]. 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]. However, release or slow-release can lead or has led to 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].

Macromolecules 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 antibacterials for dental restoratives include incorporation of a methacryloyloxydodecyl pyridinium bromide (MDPB) as an antibacterial monomer into composite resins [13], use of 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. 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 mechanical strength and antibacterial activity of commercial Fuji II LC and recently developed experimental high-strength cements.

2.3 Matrerials and Methods

2.3.1 Materials

2-dimethylaminoethanol (DMAE), bromotetradecane (BT), dipentaerythritol, 2-bromoisobutyryl bromide (BIBB), acrylic acid (AA), itaconic acid (IA), 2,2'-azobisisobutyronitrile (AIBN), triethylamine (TEA), 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). Z100 resin composite was used as received from 3M ESPE (St. Paul, MN).

2.3.2 Synthesis of the Quaternary Ammonium Aalt (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 (100 ml), BT (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 3 times. The formed 2-dimethyl-2-tetradecanyl-1-hydroxyethyl ammonium bromide (DTHAB) was then dissolved in 10% HCl aqueous solution containing a small amount of MeOH. After the solution was stirred at 110 ^{o}C for 3 h, MeOH, HBr and water were removed via a rotary evaporator. The formed 2-dimethyl-2-tetradecanyl-1-hydroxyethyl ammonium chloride (DTHAC) was purified by washing with hexane several times before drying in a vacuum oven. The synthesis scheme is shown in Figure 2.1.

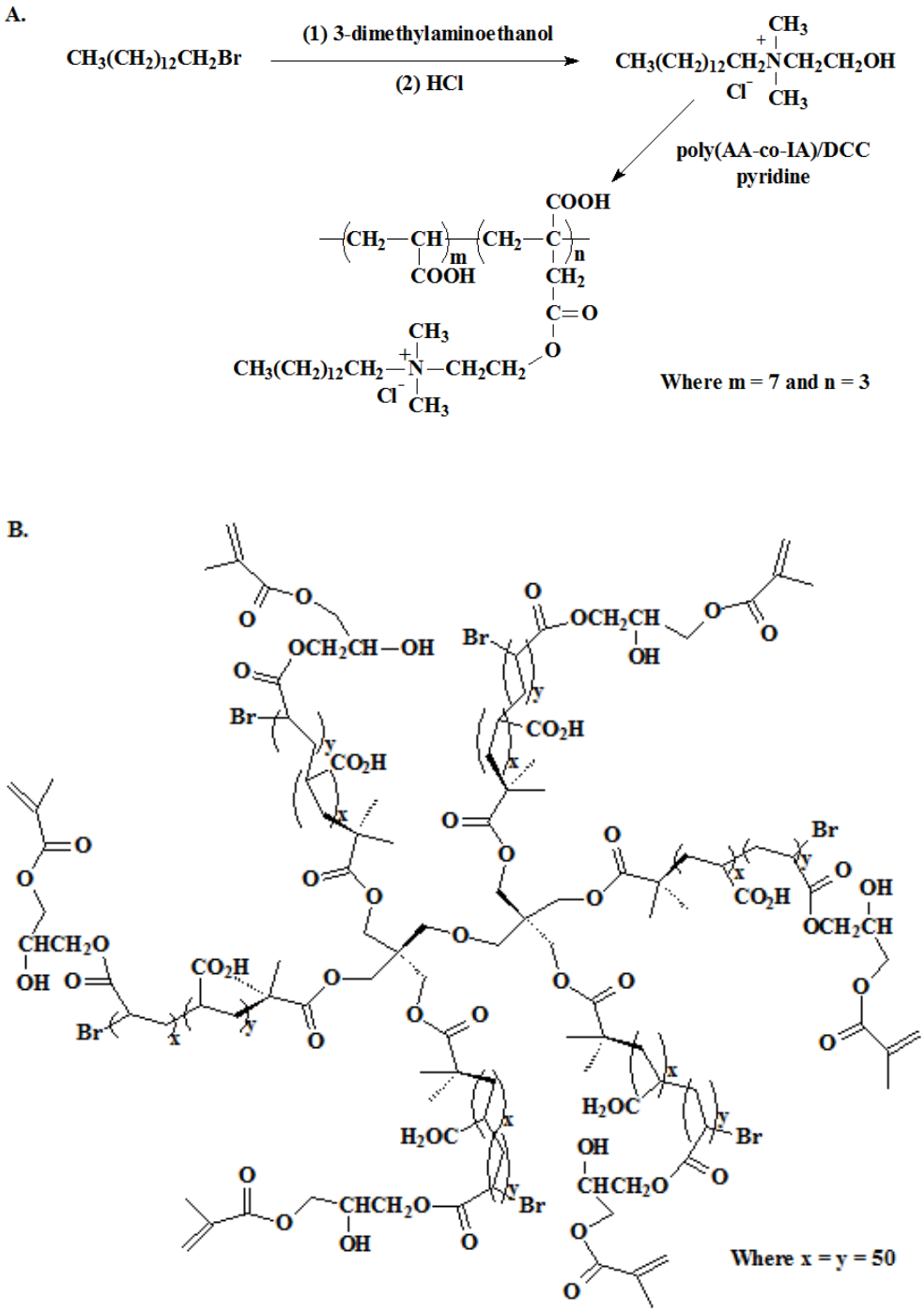


Figure 2.1 Schematic diagrams for synthesis of poly(AA-co-IA) with pendent QAS or PQAS and chemical structure of the 6-arm star-shape poly(acrylic acid) tethered with methacylate groups: (A): synthesis of PQAS; (B) chemical structure of the 6-arm star-shape poly(acrylic acid) tethered with polymerizable methacrylates.

2.3.3 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 ^{o}C for 18 h, poly(AA-co-IA) was precipitated with ether, followed by drying in a vacuum oven. Then DTHAC was tethered onto the purified poly(AA-co-IA) [21]. Briefly, to a solution of poly(AA-co-IA) in DMF, DTHAC was added with DCC and pyridine. The reaction was kept at room temperature overnight. After the insoluble dicyclohexyl urea was filtered off, the formed poly(AA-co-IA) with pendent QAS or PQAS was purified by precipitation from ether, washing with ether and drying in a vacuum oven prior to use (see Figure 2.1).

2.3.4 Synthesis of the GM-tethered Star-shape Poly(acrylic acid)

The GM-tethered 6-arm star-shape poly(acrylic acid) (PAA) 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 ℃ in the presence of CuBr (3%)-PMDETA (3%) catalyst complex via ATRP. The resultant 6-arm poly(t-BA) was hydrolyzed with HCl and dialyzed against distilled water. The purified star-shape PAA 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 PAA 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 PAA is also shown in Figure 2.1.

2.3.5 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 Corporation, 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).

2.4 Evaluation

2.4.1 Sample Preparation for Strength Tests

The experimental cements were formulated with a two-component system (liquid and powder) [22]. The liquid was formulated with the light-curable star-shape poly(acrylic acid), water, 0.9% CQ (photo-initiator, by weight) and 1.8% DC (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) = 1, 3, 5, 10, or 30% of the glass. The detailed formulations are shown in Table 2.1. Fuji II LC and Z100 were used as controls and prepared per manufacturers' instructions, where the P/L ratio = 3.2 for Fuji II LC and premixed paste for Z100.

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. 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 conditioned in 100% humidity for 15 min, removed

from the mold and conditioned in distilled water at 37 ^{o}C for 24 h unless specified, prior to testing.

2.4.2 Strength Measurements

CS and DTS 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. 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.

2.4.3 Antibacterial Test

The antibacterial test was conducted following the published procedures [23]. S. mutans (oral bacterial strain) was used for evaluation of antibacterial activity of the studied cements. Briefly, colonies of S. mutans (UA159) were suspended in 5 ml of Tryptic soy Broth (TSB), supplemented with 1% sucrose. Specimens pretreated with ethanol were incubated with S. mutans in TSB at 37 ^{o}C for 48 h under anaerobic condition with 5% CO_2. After equal volumes of the red and the green dyes 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 sec, sonicating for 10 sec as well as vortexing for another 10 sec, 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). Triple replica was used to obtain a mean value for each material.

2.4.4 Statistical Analysis

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

2.5 Results and Discussion

2.5.1 Characterization

Figure 2.2 shows the ^{1}HNMR spectra for BT, DMEA, DTHAC, poly(AA-co-IA) and poly(AA-co-IA) with pendent QAS or PQAS. The characteristic chemical shifts (ppm) are shown below: BT: 3.35 ($-CH_2Br$), 1.80 ($-CH_2CH_2Br$), 1.38 ($-CH_2-$, all) and 0.89 ($-CH_3$); DMEA: 4.40 (-OH), 3.42 ($-CH_2OH$), 2.30 ($-CH_2N-$) and 2.10 (H_3CN-); DTHAC: 5.30 (-OH), 3.82 ($-CH_2OH$), 3.35-3.45 ($-CH_2N(CH_3)_2$), 3.10 (H_3CN-), 1.65 ($-CH_2CH_2N(CH_3)_2$), 1.25 ($-CH_2-$ all) and 0.89 ($-CH_3$); poly(AA-co-IA): 12.2 (-COOH), 3.45 (-CH(COOH)-) and 1.2-2.5 ($-CH_2-$, all); 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 at the top of Figure 2 confirmed the successful attachment of DTHAC onto the poly(AA-co-IA).

Figure 2.3 shows the FT-IR spectra for BT, DMEA, DTHAC, poly(AA-co-IA) and PQAS. The characteristic peaks (cm^{-1}) are listed below: BT: 2924 (C-H stretching on $-CH_2-$), 2853 (C-H stretching on $-CH_3$), 1466, 1377 and 1251 (C-H deformation on $-CH_2-$), 721 and 647 (C-Br deformation); 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), 1040 and 776 (C-N deformation); DTHAC: 3349 and 3248 ($=N^{+}=$ stretching), 2917 (C-H stretching on $-CH_2-$), 2850 (C-H stretching on $-CH_3$), 1470 (C-H deformation on $-CH_2-$),

1090 and 730 (O-H deformation); poly(AA-co-IA): 3800-2400 (O-H stretching on –COOH), 1716 (-C=O stretching), 1196-1458 (C-H deformation on $–CH_2-$); 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), 1167-1466 (C-H deformation on $–CH_2-$) and 776 (C-N deformation). The significant peaks at 3353 for $=N^+=$ group, 2923 and 2853 for $–CH_2-$ group and 1736 for carbonyl group confirmed the formation of PQAS.

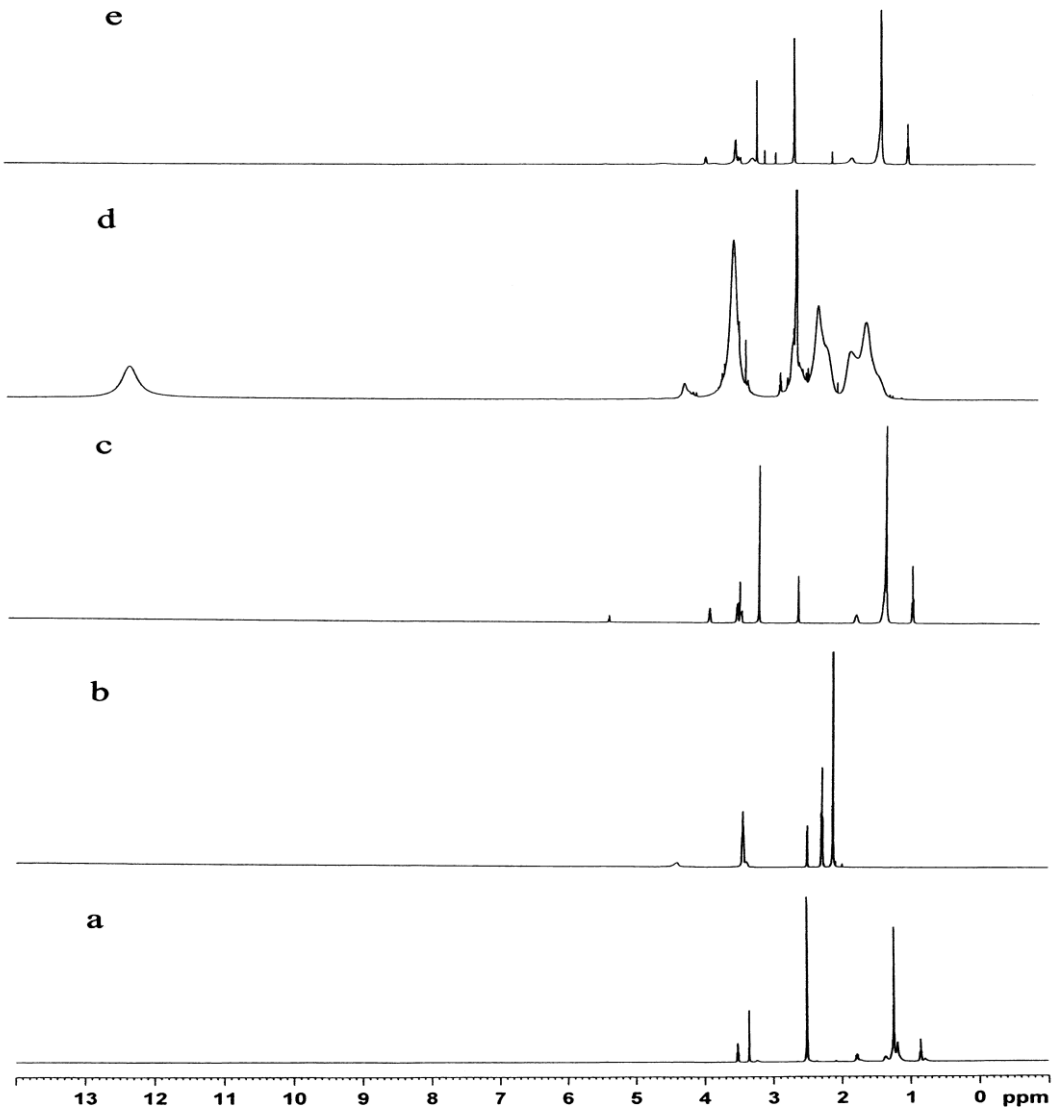


Figure 2.2 ^{1}HNMR spectra for BT, DMEA, DTHAC, poly(AA-co-IA) and PQAS: (a) BT; (b) DMEA; (c) DTHAC; (d) poly(AA-co-IA) and (e) PQAS.

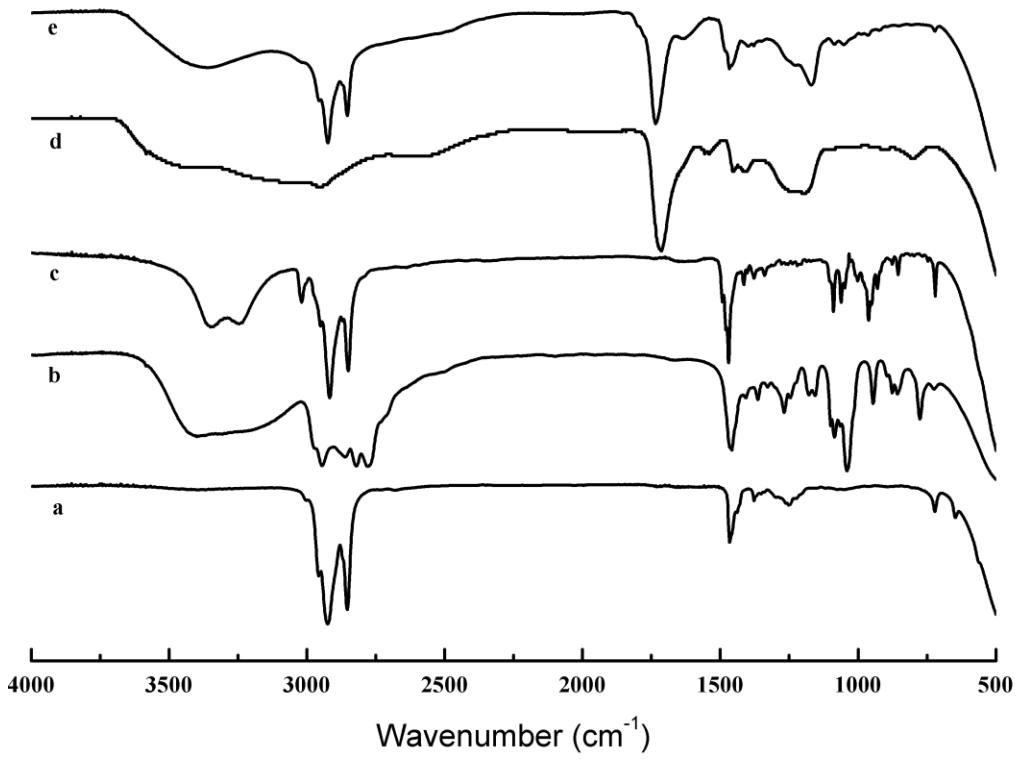


Figure 2.3 FT-IR spectra for BT, DMEA, DTHAC, poly(AA-co-IA) and PQAS: (a) BT; (b) DMEA; (c) DTHAC; (d) poly(AA-co-IA) and (e) PQAS.

2.5.2 Evaluation

Table 2.1 shows the codes, materials and formulations used in this study. Both Fuji II LC and experimental (EXPGIC) cements with and without PQAS were evaluated. PQAS was incorporated in a ratio of 1, 3, 5, 10 and 30% (by weight) of the total glass fillers.

Table 2.1 Materials and formulations used in the study

Code	Liquid formulation[1]	PQAS % (by weight)[2]	P/L ratio (by weight)[3]
FIILC	N/A	0	3.2
FIILC (1%)	N/A	1	3.2
FIILC (3%)	N/A	3	3.2
FIILC (5%)	N/A	5	3.2
FIILC (10%)	N/A	10	3.2
FIILC (30%)	N/A	30	3.2
EXP	70/30	0	2.7
EXP (1%)	70/30	1	2.7
EXP (3%)	70/30	3	2.7
EXP (5%)	70/30	5	2.7
EXP (10%)	70/30	10	2.7
EXP (30%)	70/30	30	2.7

[1]Liquid formulation: N/A = not available; Liquid for EXP = 6-arm star-shape poly(acrylic acid) vs. water (by weight); [2]PQAS = poly(AA-co-IA) with pendent QAS; PQAS was mixed with Fuji II LC filler; 0 = only Fuji II LC filler was used; [3]P/L ratio = a total amount of glass filler powder (Fuji II LC glass + PQAS) vs. polymer liquid.

Figure 2.4 shows the mean CS values of Fuji II LC and EXPGIC cements with and without PQAS addition. The CS value (MPa) was in the decreasing order of EXPGIC > EXPGIC (1%) > EXPGIC (3%) > Fuji II LC > EXPGIC (5%) > Fuji II LC (1%) > Fuji II LC (3%) > EXPGIC (10%) > Fuji II LC (5%) > Fuji II LC (10%) > EXPGIC (30%) > Fuji II LC (30%). There were no statistically significant differences between EXPGIC (3%) and Fuji II LC and between Fuji II LC (3%) and EXPGIC (10%) ($p > 0.05$). Increasing PQAS decreased the CS values of both cements. However, the decreasing rate for Fuji II LC was much faster than that for EXPGIC. With 1 to 10% PQAS addition, Fuji II LC decreased 25 to 78% of its original CS whereas EXPGIC only decreased 12 to 57%. Table 2.2 shows the results of yield strength (YS), compressive modulus, CS and DTS. The same trend was observed in Table 2.2 as shown in Figure 2.4. With 1 to 10% PQAS addition, Fuji II LC showed a decrease of 26-82% in YS, 22-78%

in modulus and 12-70% in DTS, which decreased much faster than EXPGIC (1.9-43% in YS, 2.7-34% in modulus and 1.5-43% in DTS). Figure 2.5 shows the effect of the cement aging on CS. After one month of aging in water, all the cements showed an increase in CS, especially from 1 h to 1 day. There was a slight increase (statistically no difference) for each formulation tested from 1 day to 1 week and from 1 week to 1 month.

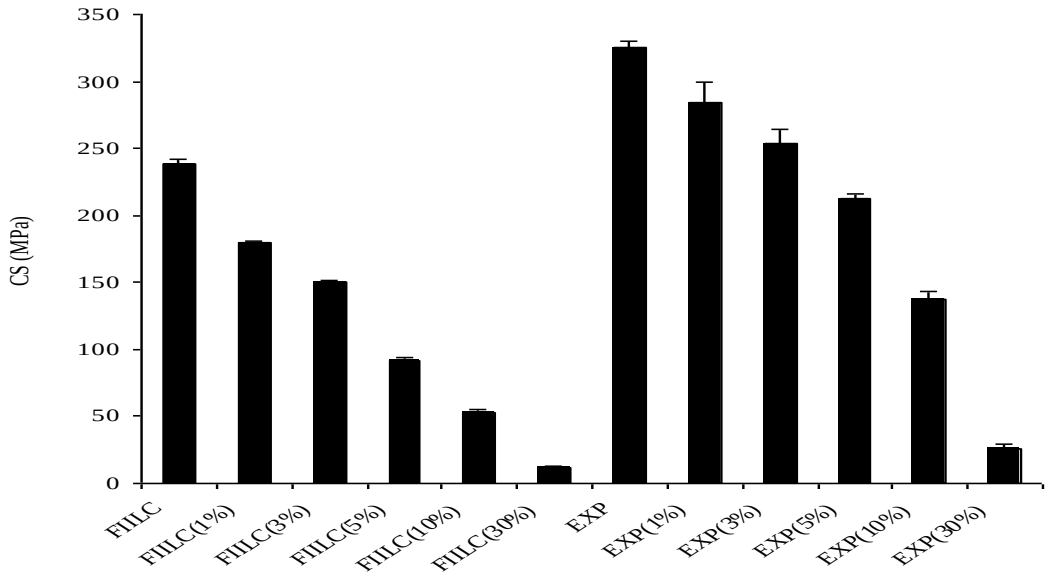


Figure 2.4 CS of Fuji II LC and experimental cements with and without PQAS addition: FIILC = Fuji II LC; EXP = EXPGIC; For Fuji II LC cements, P/L = 3.2; Filler = Fuji II LC or Fuji II LC + PQAS. For experimental cements, MW of the 6-arm poly(acrylic acid) = 17,530 Daltons; Filler = Fuji II LC or Fuji II LC + PQAS; Grafting ratio = 50%; P/L ratio = 2.7; P/W ratio = 70:30. Specimens were conditioned in distilled water at 37 ^{o}C for 24 h prior to testing.

Table 2.2 YS, modulus, CS and DTS of Fuji II LC and EXP cements

Material	YS[1] [MPa]	Modulus [GPa]	CS[2] [MPa]	DTS[3] [MPa]
FIILC	138.4 (2.2)[a, 4]	6.91 (0.42)[d]	237.9 (4.5)[g]	43.4 (4.5)
FIILC (1%)	101.3 (2.9)[b]	5.40 (0.09)[e]	179.6 (1.2)	38.3 (4.6)
FIILC (3%)	86.4 (5.2)[b]	4.53 (0.01)	149.8 (1.4)[h]	29.6 (1.8)[i]
FIILC (5%)	50.4 (2.6)	3.22 (0.24)	91.6 (2.7)	24.3 (1.5)
FIILC (10%)	24.4 (2.6)	1.54 (0.09)	52.3 (2.9)	12.9 (0.3)
EXP	173.9 (7.1)[c]	7.74 (0.04)[f]	325.3 (4.2)	58.8 (0.2)[j]
EXP (1%)	170.6 (5.5)[c]	7.53 (0.16)[f]	284.4 (15)	57.9 (2.2)[j]
EXP (3%)	173.9 (10)[c]	7.25 (0.13)[d, f]	253.7 (11)[g]	50.3 (1.7)[k]
EXP (5%)	137.7 (12)[a]	6.68 (0.08)[d]	212.0 (4.1)[g]	49.9 (3.8)[k]
EXP (10%)	98.8 (2.6)[b]	5.09 (0.09)[e]	136.9 (6.7)[h]	33.7 (2.2)[i]

[1]YS = CS at yield; [2]CS = ultimate CS; [3]DTS = diametral tensile strength; [4]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 prior to testing.

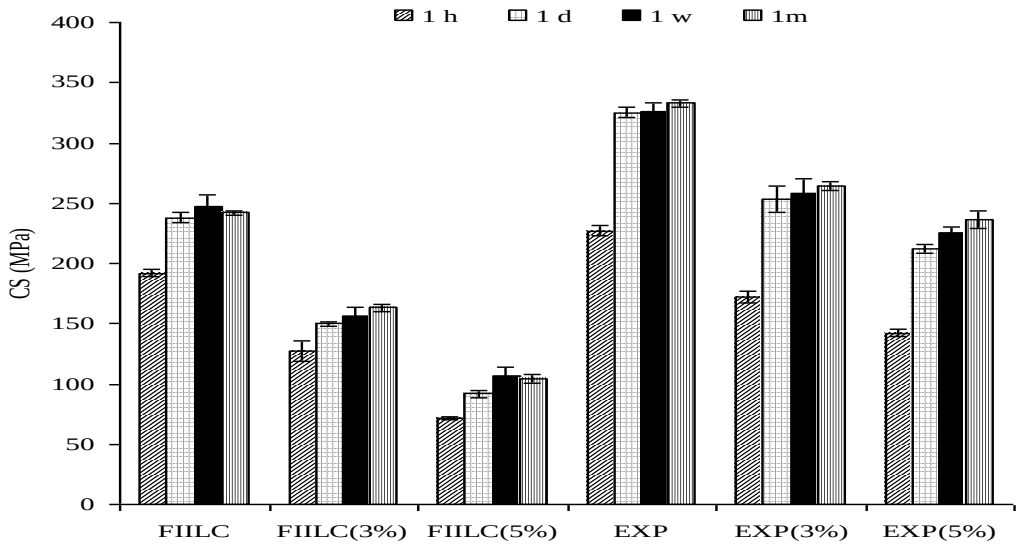


Figure 2.5 Effect of aging on CS: The formulations were the same as those described in Figure 2.4 Specimens were conditioned in distilled water at 37 °C for 1 h, 1 day, 1 week and 1 month prior to CS testing.

Figure 2.6 shows the mean S. mutans viability values after culturing with Fuji II LC and EXPGIC with and without PQAS addition. The mean S. mutans viability was in the decreasing order of Z100 > Fuji II LC > EXPGIC > Fuji II LC (1%) > EXPGIC (1%) > Fuji II LC (3%) > Fujii II LC (5%) > Fuji II LC (10%) = EXPGIC (3%) > EXPGIC (5%) > EXPGIC (10%) > Fuji II LC (30%) > EXPGIC (30%). There were no statistically significant differences among Z100, Fuji II LC and EXPGIC, among Fuji II LC (1%), Fuji II LC (3%) and EXPGIC (1%), among Fuji II LC (5%), Fuji II LC (10%) and EXPGIC (3%), and among Fuji II LC (30%), EXPGIC (5%) and EXPGIC (10%) (10%) ($p > 0.05$). Increasing PQAS decreased the S. mutans viability. With 3 to 30% PQAS addition, Fuji II LC killed 45 to 79% of S. mutans whereas EXPGIC killed 63 to 91%, indicating that the killing power of EXPGIC was much higher than that for Fuji II LC. Figure 2.7 shows the effect of the cement aging on the S. mutans viability. No significant changes in the S. mutans viability were found for each formulation tested except Fuji II LC and EXP, where the S.mutans viability was significantly higher in 1 day than in either 3 days or 1 week ($p > 0.05$).

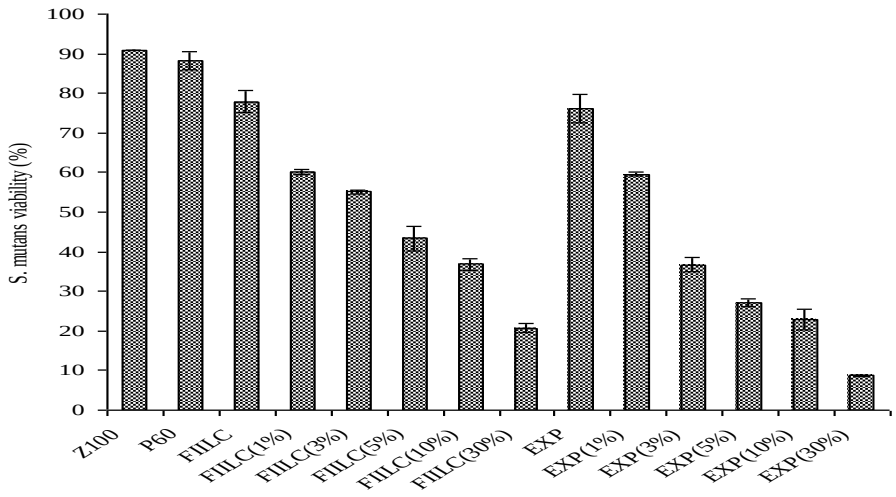


Figure 2.6 The S. mutans viability after culturing with Fuji II LC and experimental cements with and without PQAS addition: The formulations were the same as those described in Figure 4. Specimens were conditioned in distilled water at 37 ^{o}C for 24 h, followed by incubating with S. mutans before antibacterial testing.

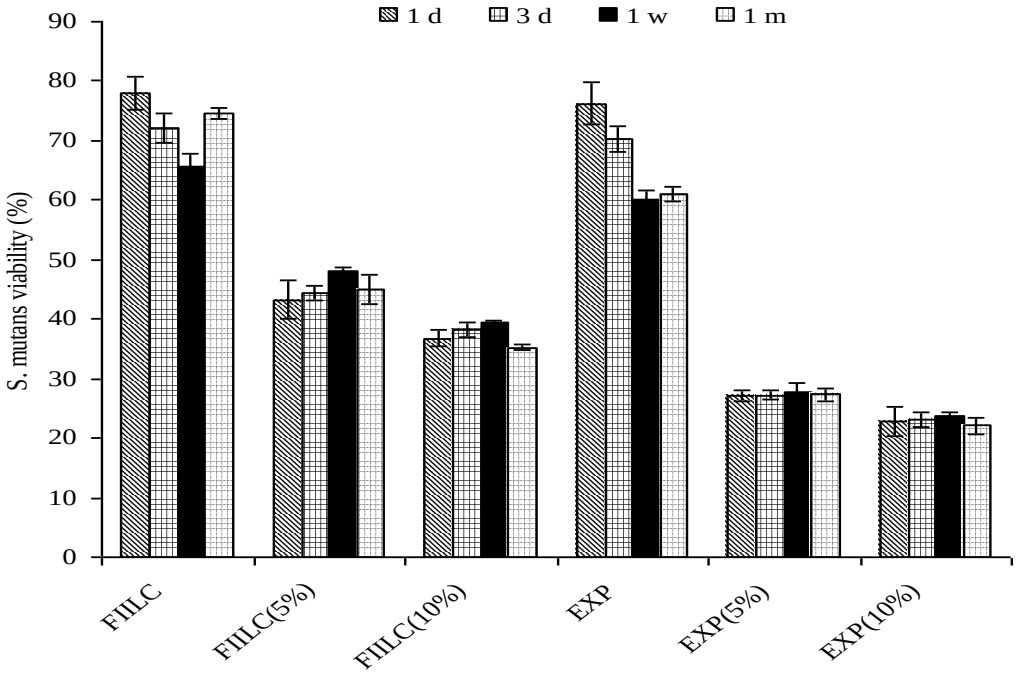


Figure 2.7 Effect of aging on the S. mutans viability after culturing with Fuji II LC and experimental cements with and without PQAS addition: The formulations were the same as those described in Figure 5. The specimens were conditioned in distilled water for 1 day, 3 days, 1 week and 1 month, followed by incubating with S. mutans before antibacterial testing.

2.5.3 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]. 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 beauty of using QAS is that they can kill the microorganism by touch or 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 MW [15]. In this regard, we purposely synthesized the new PQAS, incorporated it into both Fuji II LC and our experimental high-strength cements and evaluated the CS and antibacterial function of the formed cements.

From the results in Figure 2.4 and Table 2.2, apparently both Fuji II LC and EXPGIC cements showed a decrease in CS, YS, modulus and DTS with increasing PQAS. This can be attributed to the reason that the incorporated QAS contains a 14 carbon long chain that does not contribute to any strength enhancement. On the other hand, EXPGIC showed a slower decreasing pace with nearly 30% less in CS decrease as compared to Fuji II LC (see Figure 2.4). This result implies that there may be some strong intermolecular interactions between PQAS and star-shape polymers. Furthermore, EXPGIC still kept its CS above 200 MPa at PQAS = 5% or less, which may be attributed to its original high strength (325 MPa).

Regarding the antibacterial activity, we also tested a commercial dental composite resin Z100 for comparison. We found that Z100 hardly killed S. mutans. After 48 h incubation with S. mutans, Z100 only killed 10% S. mutans (see Figure 2.5). Composite resins usually do not have antibacterial functions [5, 6]. Both Fuji II LC and EXPGIC cements without PQAS addition killed about 20% S. mutans, 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 function significantly. More interestingly, EXPGIC showed an even stronger antibacterial activity than Fuji II LC with 3 to 30% PQAS addition. The possible reason may be explained below. Since PQAS is composed of 50% carboxylic acid and 50% QAS and both components are very hydrophilic, they like to have interactions with other hydrophilic components from the cement in the presence of water. EXPGIC contains only hydrophilic GM-tethered poly(acrylic acid) (70%) and water (30%), whereas Fuji II LC contains a substantial amount (approximately 25-35%) of 2-hydroxyethyl methacrylate

(partially hydrophilic) and dimethacrylaye/oligomethacrylte (very hydrophobic), except for the linear poly(acrylic acid) (20-30%) and water (20-30%) [24]. Therefore, the components in EXPGIC may help the PQAS chains better extend on the surface of the cements but dimethacrylaye/oligomethacrylte and 2-hydroxyethyl methacrylate in Fuji II LC may restrict or interfere with the extension of the PQAS chains on the surface. Obviously, 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 Fuji II LC. This outcome is very encouraging because it will allow us to use the minimum amount of PQAS in EXPGIC to obtain the maximum antibacterial activity without significantly reducing mechanical strengths.

As previously discussed, 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 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 leachable. 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 2.5 showed that there was a slight increase in CS for all the formulations tested after one month of aging, indicating no PQAS leaching. The result in Figure 2.7 showed that there was no change or reduction in antibacterial function for all the formulations tested, also suggesting no leaching. Otherwise, both strength and antibacterial function would decrease with aging. The reason can be attributed to the fact that the PQAS 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 synthesized in the study not only provided QAS for antibacterial function but also supplied carboxyl groups for salt-bridge formation. The latter helped the PQAS polymer firmly attached to the glass fillers.

2.6 Conclusions

We have developed a novel antibacterial glass-ionomer cement containing non-leachable PQAS. With PQAS addition, both Fuji II LC and experimental cements showed a reduction in CS with 25-95% for Fuji II LC and 13-78% for the experimental cement and a reduction in S. mutans viability with 40-79% for Fuji II LC and 40-91% for the experimental cement. The experimental cement showed less CS reduction and higher antibacterial activity as compared to Fuji II LC. The result also indicates that the cements are permanently antibacterial with no PQAS leaching. 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 permanent antibacterial function.

3. SYNTHESIS AND EVALUATION OF AN ANTIBACTERIAL DENTAL CEMENT CONTAINING QUATERNARY AMMONIUM BROMIDES

3.1 Abstract

The objective of this study was to synthesize the poly(quaternary ammonium salt) (PQAS)-containing polymer, use the polymer to formulate the light-curable glass-ionomer cements, and evaluate the mechanical strengths and S. mutans viability of the formed cements. The functional QAS and their constructed PQAS were synthesized, characterized and formulated into both Fuji II LC and experimental high-strength cements. Compressive strength (CS) and S. mutans viability were used to evaluate the mechanical strength and antibacterial activity of the cements. Fuji II LC cement was used as control. The specimens were conditioned in distilled water at 37 ^{o}C for 24 h prior to testing. The effects of the substitute chain length, loading as well as grafting ratio of the QAS and aging on CS and cell viability were investigated. The results show that all the PQAS-containing cements showed a significant antibacterial activity, accompanying with a CS reduction. The effects of the chain length, loading and grafting ratio of the QAS were significant. Increasing chain length, loading, grafting ratio significantly enhanced antibacterial activity but reduced CS. The experimental cement showed less CS reduction and higher antibacterial activity as compared to Fuji II LC. The long-term aging study indicates that the cements are permanently antibacterial with no PQAS leaching. There was no significant difference between QAS bromide and QAS chloride, suggesting that we can use QAS bromide directly without converting bromide to chloride. 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 permanent antibacterial function.

3.2 Introduction

Long-lasting restoratives and restoration are clinically attractive because they can reduce patents' pain and expense as well as the number of their visits to dental offices [1-4]. It is known that both restorative materials and oral bacteria are mainly responsible for the restoration failure [2]. 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 in the presence of fermentable carbohydrates [4]. 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]. 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].

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 antibacterials for dental restoratives include incorporation of a methacryloyloxydodecyl pyridinium bromide (MDPB) as an antibacterial monomer into composite resins [13], use of 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. 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.

3.3 Materials and Methods

3.3.1 Materials

2-dimethylaminoethanol (DMAE), bromoethane, bromohexane, bromodecane, bromdodecane, bromotetradecane, bromohexadecane, dipentaerythritol, 2-bromoisobutyryl bromide (BIBB), acrylic acid (AA), itaconic acid (IA), 2,2'-azobisisobutyronitrile (AIBN), triethylamine (TEA), 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). Z100 resin composite was used as received from 3M ESPE (St. Paul, MN).

3.3.2 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 (100 ml), 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 3 times. The formed 2-dimethyl-2-hexyl-1-hydroxyethyl ammonium bromide (or namely 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 3.1 A.

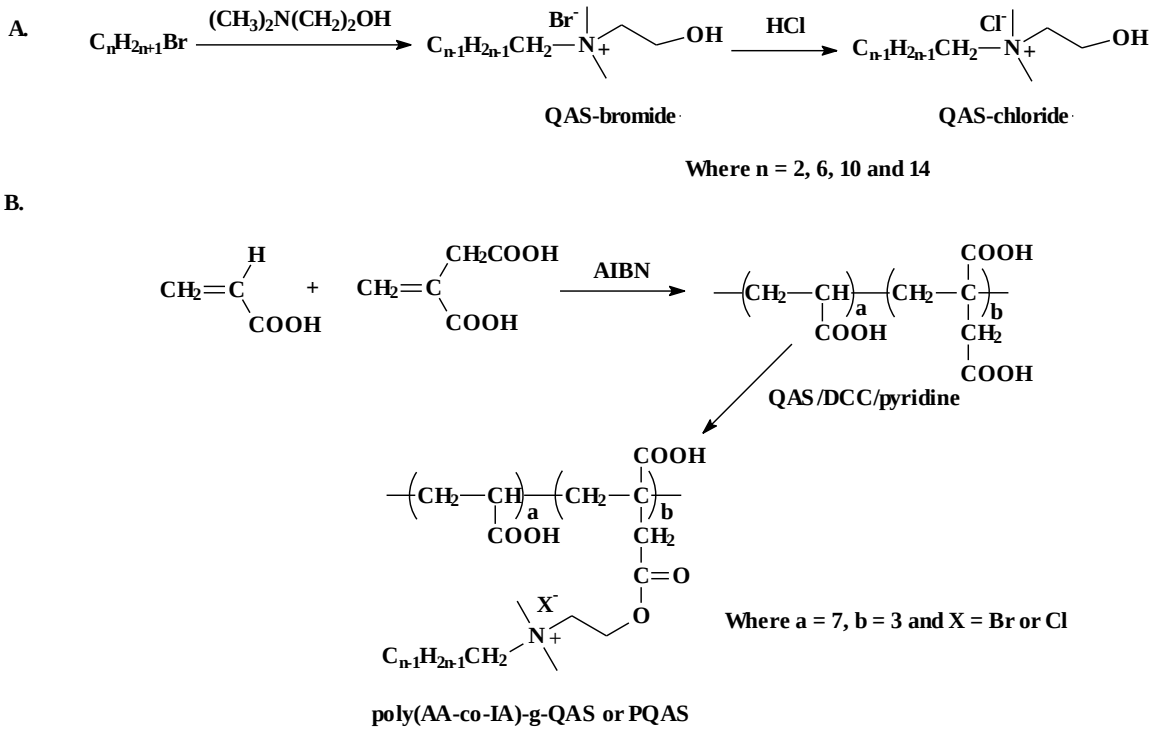


Figure 3.1 Synthesis scheme for QAS and PQAS: (A) synthesis of QAS; (B) synthesis of poly(AA-co-IA), followed by tethering QAS onto the polymer.

3.3.3 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 ^oC 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 the 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 Figure 3.1 B).

3.3.4 Synthesis of the GM-tethered Star-shape Poly(acrylic acid)

The GM-tethered 6-arm star-shape poly(acrylic acid) (PAA) 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 ℃ in the presence of CuBr (3%)-PMDETA (3%) catalyst complex via ATRP. The resultant 6-arm poly(t-BA) was hydrolyzed with HCl and dialyzed against distilled water. The purified star-shape PAA 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 PAA 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 PAA is also shown in Figure 3.2.

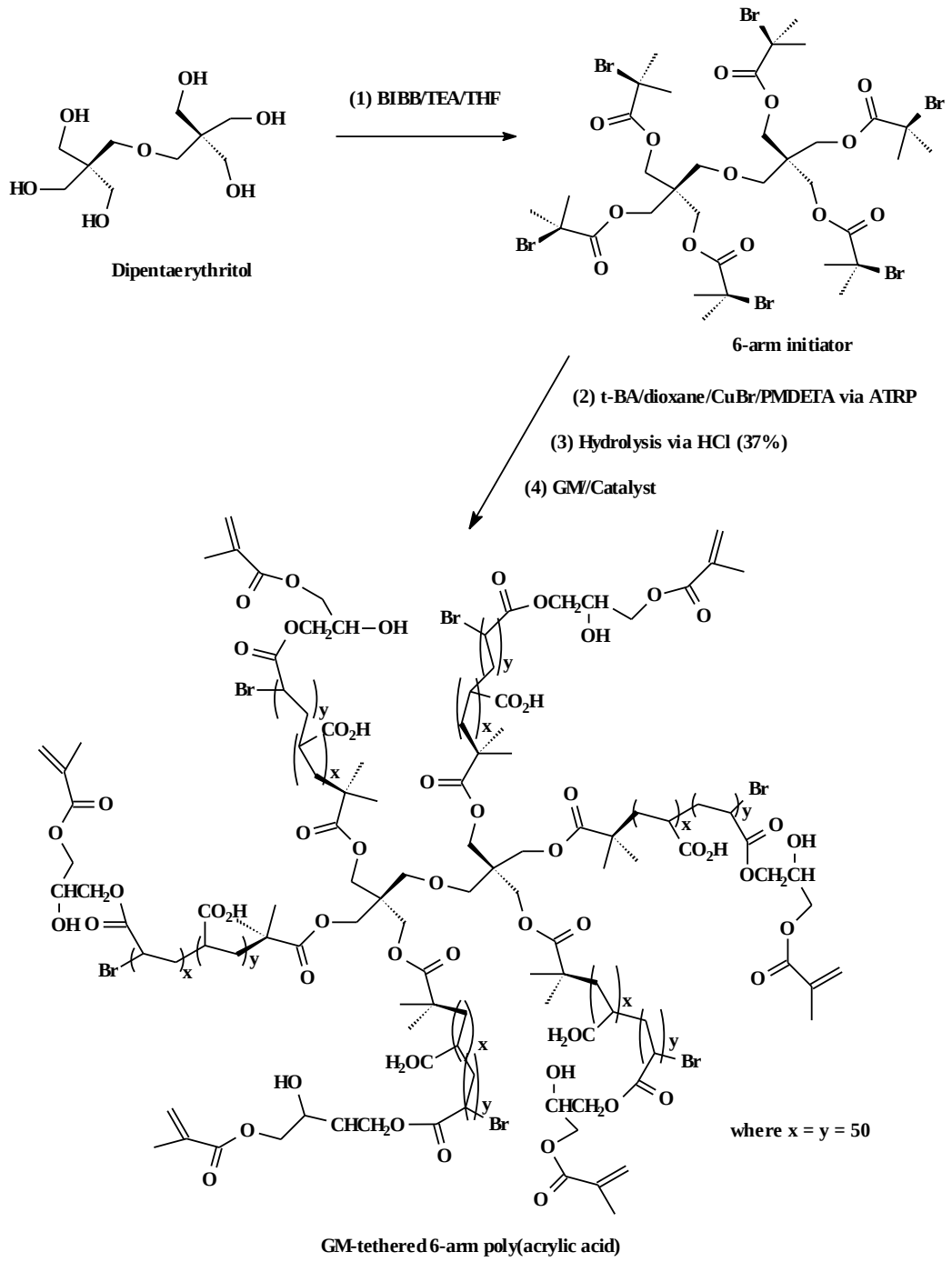


Figure 3.2 Synthesis scheme for star-shape poly(acrylic acid): synthesis of the 6-arm star-shape poly(acrylic acid) tethered with polymerizable methacrylates.

3.3.5 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 Corporation, 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).

3.4 Evaluation

3.4.1 Sample Preparation for Strength Tests

The experimental cements were formulated with a two-component system (liquid and powder) [22]. The liquid was formulated with the light-curable star-shape poly(acrylic acid), water, 0.9% CQ (photo-initiator, by weight) and 1.8% DC (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) = 1, 3, 5, 10, or 30% of the glass. The detailed formulations are shown in Table 3.1. Fuji II LC was used as control and prepared per manufacturer's instruction where the 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 conditioned in 100% humidity for 15 min, removed from the mold and conditioned in distilled water at 37 ^{o}C for 24 h unless specified, prior to testing.

3.4.2 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 and 16 mm, respectively, 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.

3.4.3 MIC Test for the Synthesized QAS

The minimal inhibitory concentration (MIC) of the synthesized QAS was determined following the published protocol with a slight modification. 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 placing in 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 x 10^4 µg/ml. The microtiter plate was then inoculated with S. mutans suspension (cell concentration = 5 x 10^5 CFU/ml) and incubated at 37 ^{o}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.

3.4.4 Antibacterial Test for the Formed Cements

The antibacterial test was conducted following the published procedures [23]. S. mutans (oral bacterial strain) was used for evaluation of antibacterial activity of the studied cements. Briefly, colonies of S. mutans (UA159) were suspended in 5 ml of Tryptic soy Broth (TSB), supplemented with 1% sucrose, to make a suspension with 10^8 CFU/ml of S. mutans, after 24 h incubation. Specimens pretreated with ethanol (10 sec) were incubated with S. mutans in TSB at 37 ^oC for 48 h under anaerobic condition with 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) 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 sec, sonicating for 10 sec as well as vortexing for another 10 sec, 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). Triple replica was used to obtain a mean value for each material.

3.4.5 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.

3.5 Results and Discussion

3.5.1 Characterization

The characteristic chemical shifts (ppm) from the ^{1}HNMR spectra (see Figure 3.3) for bromohexane, DMEA, B6, C6, poly(AA-co-IA) and poly(AA-co-IA) with pendent

QAS or 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) C6: 5.35 (-OH), 3.58 (-CH_2OH), 3.20 (-$CH_2N(CH_3)_2$), 2.90 (H_3CN-), 1.45 (-$CH_2CH_2N(CH_3)_2$), 1.025 (-CH_2- all) and 0.62 (-CH_3); (e) poly(AA-co-IA): 12.2 (-COOH), 3.45 (-CH(COOH)-) and 1.2-2.5 (-CH_2-, all); (f) 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 chemical shifts for C6 appeared in a lower position than those for B6.

The characteristic peaks (cm^{-1}) from the FT-IR spectra (see Figure 3.4) for bromohexane, DMEA, B6, C6, 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-), 721 and 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), 1040 and 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-), 1090 and 730 (O-H deformation); (d) C6: 3600-3200 (=N^+= stretching), 2909 (C-H stretching on -CH_2-), 2843 (C-H stretching on -CH_3), 1459 (C-H deformation on –CH_2-), 1090 and 730 (O-H deformation); (e) poly(AA-co-IA): 3800-2400 (O-H stretching on –COOH), 1716 (-C=O stretching), 1196-1458 (C-H deformation on –CH_2-); (f) 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), 1167-1466 (C-H deformation on –CH_2-) and 776 (C-N deformation). 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.

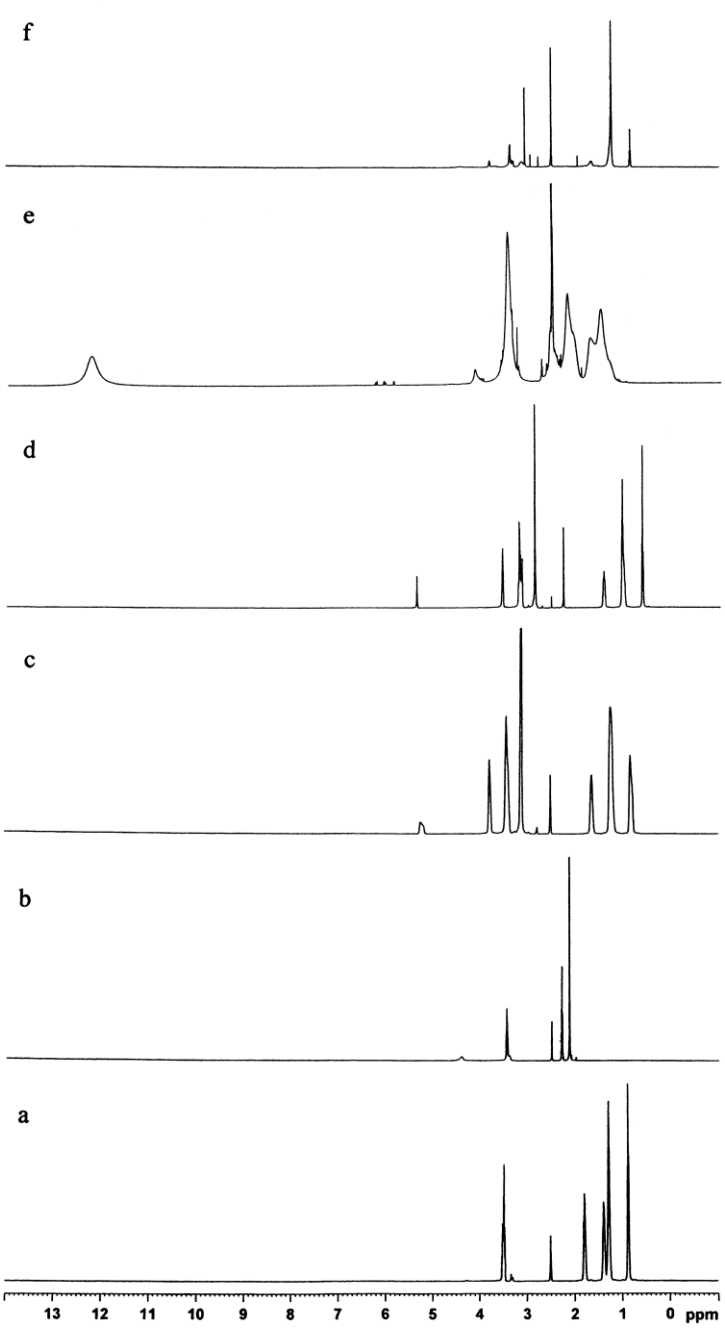


Figure 3.3 ^{1}HNMR spectra for bromohexane, DMEA, B6, C6, poly(AA-co-IA) and PQAS: (a) bromohexane; (b) DMEA; (c) B6; (d) C6; (e) poly(AA-co-IA) and (f) PQAS.

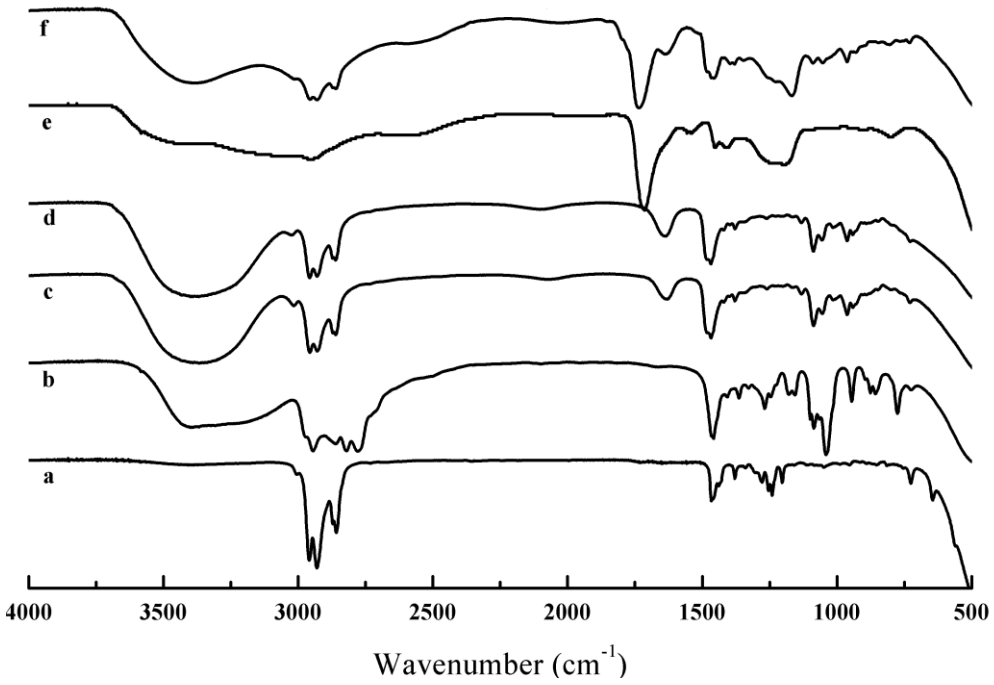


Figure 3.4 FT-IR spectra for bromohexane, DMEA, B6, C6, poly(AA-co-IA) and PQAS: (a) bromohexane; (b) DMEA; (c) B6; (d) C6; (e) poly(AA-co-IA) and (f) PQAS.

3.5.2 Evaluation

Table 3.1 shows the code, description and MIC of the synthesized QAS. The MIC values ranged from 1.563 to 2 x 10^4 µg/ml for B16 to B2 and 1.563 to 100 µg/ml for C16 to C10.

Table 3.1 Codes, description, MIC values of the synthesized QAS

Code	QAS[1]	Chain length	MIC (μg/ml)[2]
B2	2-Dimethyl-2-ethyl-1-hydroxyethylammonium bromide	2	20,000
B6	2-Dimethyl-2-hexyl-1-hydroxyethylammonium bromide	6	1,000
B10	2-Dimethyl-2-decyl-1-hydroxyethylammonium bromide	10	200
B12	2-Dimethyl-2-dodecyl-1-hydroxyethylammonium bromide	12	25
B14	2-Dimethyl-2-tetradecyl-1-hydroxyethylammonium bromide	14	3.125
B16	2-Dimethyl-2-hexadecyl-1-hydroxyethylammonium bromide	16	1.563
C10	2-Dimethyl-2-decyl-1-hydroxyethylammonium chloride	10	100
C12	2-Dimethyl-2-dodecyl-1-hydroxyethylammonium chloride	12	25
C14	2-Dimethyl-2-tetradecyl-1-hydroxyethylammonium chloride	14	1.563
C16	2-Dimethyl-2-hexadecyl-1-hydroxyethylammonium chloride	16	1.563

[1]All the QAS were freshly synthesized and were water-soluble; [2]MIC values were measured as described in the text.

Table 3.2 shows the effect of the substitute chain length of the synthesized QAS on CS and S. mutans viability of both Fuji II LC and EXPGIC cements. 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), where there were no statistically significant differences among Fuji II LC (B2), Fuji II LC (B6), Fuji II LC (B10) and Fuji II LC (B14), between EXPGIC (B2) and EXPGIC (B6), and between EXPGIC (B10) and EXPGIC (B14) ($p > 0.05$). 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), where there were

no statistically significant differences among Fuji II LC and EXPGIC, between Fuji II LC (B2) and Fuji II LC (B6), among Fuji II LC (B6), Fuji II LC (B10), Fuji II LC (B14) and EXPGIC (B2), between Fuji II LC (B14) and EXPGIC (B6), and Between EXPGIC (B10) and EXPGIC (B14) ($p > 0.05$). The PQAS addition significantly decreased the S. mutans viability, with a reduction of 38-56% for Fuji II LC and 52-73% for EXPGIC. Increasing the substitute chain length on the QAS decreased the S. mutans viability of both cements but the decreasing rate was not as dramatic as the MIC values for the QAS in water (see Table 3.1). Moreover, the PQAS in EXPGIC showed a higher killing power to S. mutans than those in Fuji II LC.

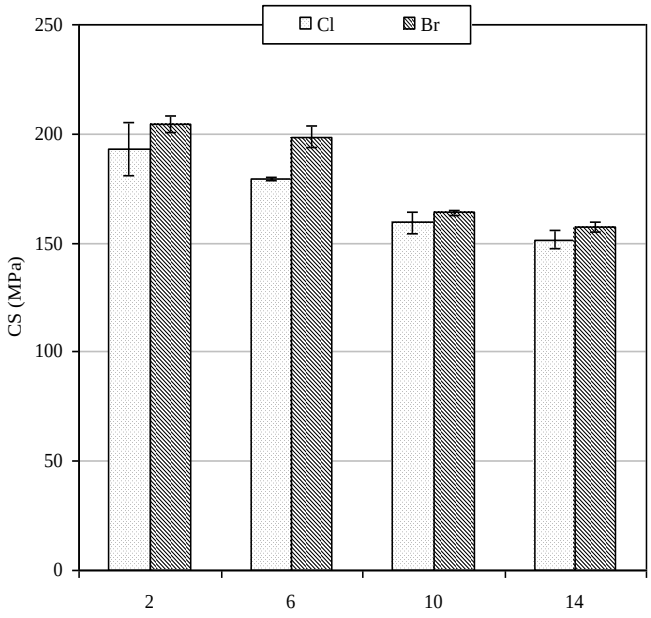
Figure 3.5 shows the effect of PQAS bromide and PQAS chloride on CS and S. mutans viability of EXPGIC. Like those in Table 3.2, increasing the substitute chain length on the QAS decreased the CS values of the cements and S. mutans viability. Although it seems that some of them were not statistically significant from each other, the cements formulated with PQAS bromide showed less CS reduction (see Figure 3.5a) but lower S. mutans viability (Figure 3.5b) than those with PQAS chloride. The results suggest that PQAS bromide is better than PQAS chloride.

Figure 3.6 shows the effect of the PQAS loading on CS and S. mutans viability of EXPGIC. Increasing the PQAS loading decreased CS and S. mutans viability of EXPGIC. With 1 to 20% PQAS loading, EXPGIC lost 18 to 80% of its original CS but killed 17 to 83% of S. mutans correspondingly. The more the PQAS added, the lower the CS and S. mutans viability. Figure 3.7 shows the effect of the QAS-grafting ratio on poly(AA-co-IA) on CS and S. mutan viability. Like the trend shown in Figure 3.7, increasing the QAS-grafting ratio decreased CS and S. mutans viability of EXPGIC. With increasing the QAS-grafting ratio from 10 to 70%, EXPGIC lost 13 to 51% of its original CS but killed 52 to 71% of S. mutans correspondingly.

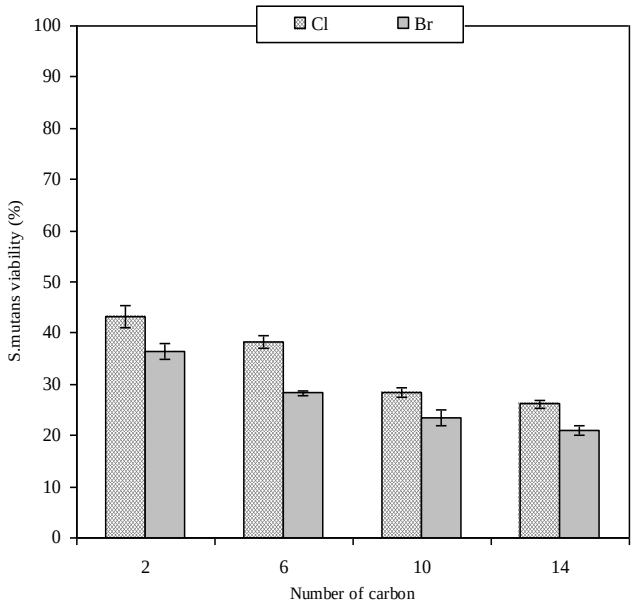
Table 3.2 Effect of the chain length of the synthesized QAS on CS and S. mutans viability of the cements

Material[1]	Chain length[2]	CS [MPa]	S. mutans viability [%]
FIILC	---	237.9 (4.5)[3]	77.9 (2.7)[d]
FIILC(B2)	2	117.8 (1.3)[a]	48.1 (2.8)[e]
FIILC(B6)	6	112.3 (2.1)[a]	43.7 (1.3)[e, f]
FIILC(B10)	10	96.1 (2.8)[a]	37.7 (1.4)[f]
FIILC(B14)	14	95.9 (3.7)[a]	34.4 (1.3)[f, g]
EXP	---	325.3 (4.2)	76.2 (3.5)[d]
EXP(B2)	2	204.1 (3.8)[b]	36.4 (1.5)[f]
EXP(B6)	6	198.7 (4.7)[b]	28.3 (0.5)[g]
EXP(B10)	10	163.8 (1.2)[c]	23.4 (1.6)[h]
EXP(B14)	14	157.5 (1.2)[c]	20.9 (0.9)[h]

[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 QAG grafting ratio = 50%. For Fuji II LC cements, P/L = 3.2; For experimental cements, MW of the 6-arm poly(acrylic acid) = 17,530 Daltons; 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 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 ^{o}C for 24 h, followed by direct testing for CS or/and incubating with S. mutans for 48 h for antibacterial testing.



(a)



(b)

Figure 3.5 Effect of PQAS bromide and PQAS chloride on CS and S. mutans viability of EXPGIC: (a) The effect on CS; (b) The effect on the S. mutans viability. The description on QAS and the formulations of the cements can be found in Tables 1 and 2, respectively. Specimens were conditioned in distilled water at 37 ^{o}C for 24 h, followed by direct testing for CS or/and incubating with S. mutans for 48 h for antibacterial testing.

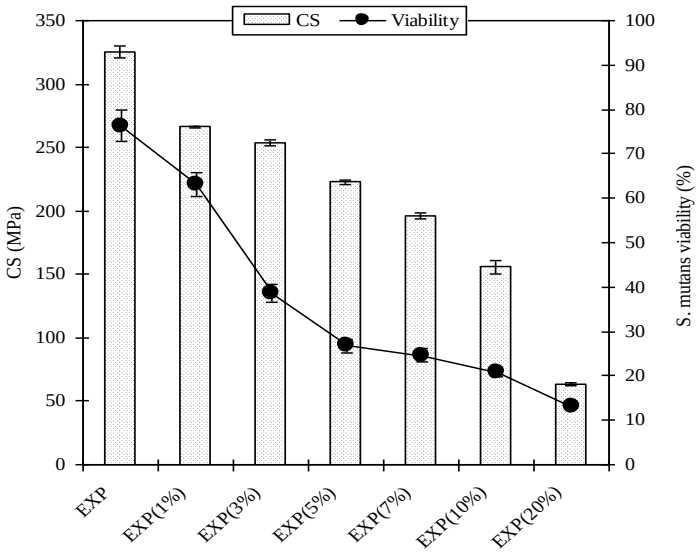


Figure 3.6 Effect of PQAS content on both CS and S. mutans viability of EXPGIC: The formulations were the same as those described in Figure 3.5, except for PQAS content change and QAS = B6 (see Table 3.2). Specimens were conditioned in distilled water at 37 ^{o}C for 24 h, followed by direct testing for CS or/and incubating with S mutans for 48 h for antibacterial testing.

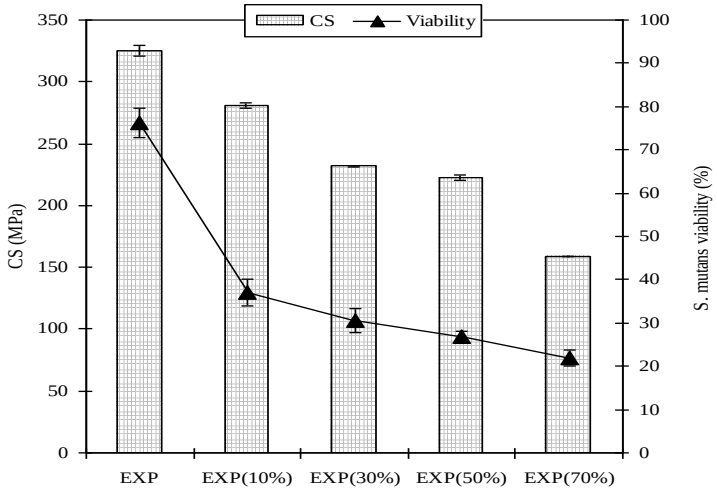


Figure 3.7 Effect of QAS-grafting ratio on poly(AA-co-IA) on both CS and S. mutans viability: The formulations were the same as those described in Figure 3.6, except for PQAS content = 5%. Specimens were conditioned in distilled water at 37 ^{o}C for 24 h, followed by direct testing for CS or/and incubating with S. mutans for 48 h for antibacterial testing.

Figure 3.8 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 3.3 shows the property comparison among Fuji II LC, EXPGIC and EXPGIC (B6) with 5% PQAS loading and 50% QAS-grafting. For YS, M and UCS or CS, the mean strength values were in the decreasing order of EXPGIC > Fuji II LC > EXPGIC-PQAS, where there was no statistically significant difference between EXPGIC-PQAS and Fuji II LC for UCS ($p > 0.05$). For DTS and FS, EXPGIC > EXPGIC-PQAS > Fuji II LC, where there were no significant differences between EXPGIC-PQAS and Fuji II LC for either DTS or FS ($p > 0.05$). For the S. mutans viability, Fuji II LC > EXPGIC > EXPGIC-PQAS, where there was no significant difference between EXPGIC and Fuji II LC ($p > 0.05$).

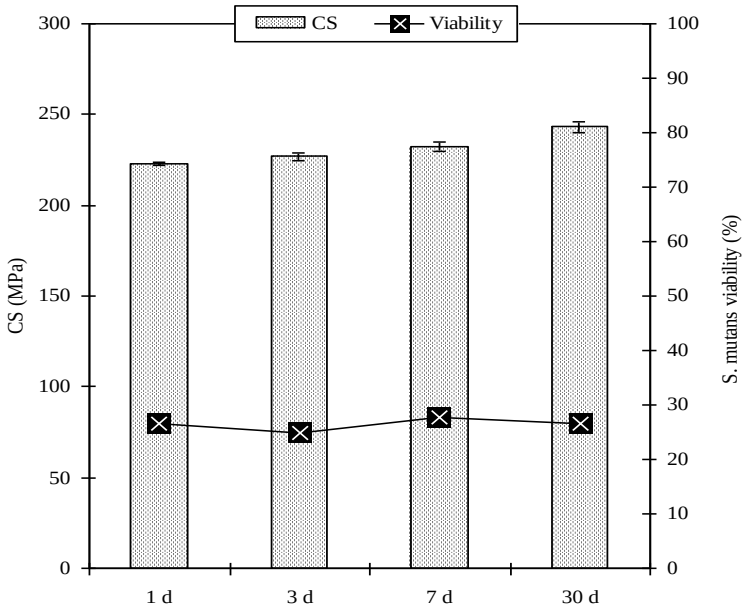


Figure 3.8 Effect of aging on both CS and S. mutans viability after culturing with experimental cements with and without PQAS addition: The formulations were the same as those described in Figure 3.7, except for QAS grafting ratio = 50%. The specimens were conditioned in distilled water for 1 day, 3 days, 1 week and 1 month, followed by direct testing for CS and incubating with S. mutans for 48 h for antibacterial testing.

Table 3.3 Comparison of properties of Fuji II LC, EXPGIC and PQAS-containing EXPGIC

Material	YS[1] [MPa]	M[2] [GPa]	UCS[3] [MPa]	DTS[4] [MPa]	FS	Viability (%)
Fuji II LC	138.4 (2.2)[5]	6.91 (0.42)	237.9 (4.5)[a]	43.4 (4.5)[b]	52.8 (1.9)[c]	77.9 (2.7)[d]
EXPGIC	173.9 (7.1)	7.74 (0.04)	325.3 (4.2)	58.8 (0.2)	88.3 (2.5)	76.2 (3.5)[d]
EXPGIC-PQAS	110.2 (6.0)	5.62 (0.08)	222.8 (1.9)[a]	49.8 (1.2)[b]	59.9 (2.3)[c]	26.7 (0.9)

[1]YS = CS at yield; [2]M = compressive modulus; [3]UCS = ultimate CS; [4]DTS = diametral tensile strength; [5]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 ^{o}C for 24 h, followed by direct testing for CS or/and incubating with S. mutans for 48 h for antibacterial testing.

3.5.3 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]. 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 beauty of using QAS is that they can kill the microorganism by touch or 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 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 function 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 1, 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 and C16) 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. There were no significant differences in biocidal activity between QAS bromide and QAS chloride.

From the results in Table 3.2, with PQAS addition both Fuji II LC and EXPGIC cements showed a decrease in CS and S. mutans viability. 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 the S. mutans viability, we found that both Fuji II LC and EXPGIC cements without PQAS addition killed about 20% S. mutans, 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 function 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 like to have interactions with other hydrophilic components from the cement in the presence of water. By comparing the compositions in polymer liquid, EXPGIC contains only hydrophilic GM-tethered poly(acrylic acid) (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(acrylic acid) (20-30%) and water (20-30%) [24]. 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. 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.

QAS can be made either as QAS bromide or QAS chloride. We hypothesized that QAS chloride might favor the mechanical strength as compared to QAS bromide due to the smaller size of chlorine. However the results in Table 3.1 showed that QAS bromide was similar to QAS chloride in inhibition of S. mutans based on MIC test. The results in Figure 3.4 and Figure 3.5 also showed that the PQAS bromide-composed cements were better in mechanical strength and antibacterial activity than those containing PQAS chlorine. The results rejected our hypothesis and suggest that it is not necessary to convert QAS bromide to QAS chloride. We can use QAS bromide directly to develop the antibacterial cements.

The effect of the QAS (B6) loading on CS and S. mutans activity of EXPGIC is shown in Figure 3.6 Apparently, the more the synthesized PQAS added the lower the CS values and the higher the antibacterial activity. To keep the CS value above 200 MPa and S. mutans viability 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 S. mutans viability. The result in Fig. 3.7 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 S. mutans viability 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 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 Fig. 3.8 shows that there was a slight increase in CS but no change or no reduction in antibacterial function after one month of aging, indicating that there was 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 not only provided QAS for antibacterial function but also supplied carboxyl groups for salt-bridge formation. The latter helped the PQAS polymer firmly attached to the glass fillers. Therefore, we developed a GIC with permanent antibacterial function.

Finally we compared yield strength (YS), compressive modulus (M), ultimate CS (or CS), diametral tensile strength (DTS), flexural strength (FS) and S. mutans viability of the experimental cement having the optimal formulation with those of both EXPGIC and Fuji II LC without any PQAS addition. The PQAS-containing experimental cement was 37% in YS, 27% in modulus, 31% in CS, 15% in DTS and 32% in FS lower than EXPGIC without PQAS addition and 20% in YS, 18% in modulus and 6% in CS lower but 15% in DTS and 13% in FS higher than Fuji II LC. Furthermore, the experimental

cement with PQAS addition was much higher in antibacterial activity than both EXPGIC (65% higher) and Fuji II LC (66% higher).

3.6 Conclusions

A novel PQAS-containing antibacterial glass-ionomer cement with permanent antibacterial activity has been developed. 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 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. There was no significant difference between QAS bromide and QAS chloride, suggesting that we can use QAS bromide directly without converting bromide to chloride. The experimental cement showed less CS reduction and higher antibacterial activity than Fuji II LC. The long-term aging study indicates that the cements are permanently antibacterial 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 permanent antibacterial function.

4. CONCLUSION

We have developed a novel PQAS-containing antibacterial glass-ionomer cement with permanent antibacterial function. 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 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. There was no significant difference between QAS bromide and QAS chloride, suggesting that we can use QAS bromide directly without converting bromide to chloride. The experimental cement showed less CS reduction and higher antibacterial activity than Fuji II LC. The long-term aging study indicates that the cements are permanently antibacterial 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 permanent antibacterial function.

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