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A new antibacterial bone cement modified with quaternary ammonium bromide-composed polymers

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***Abstract*— A new quaternary ammonium bromide-containing antibacterial bone cement has been developed and evaluated. A quaternary ammonium bromide-methyl methacrylate copolymer was synthesized and incorporated into a traditional poly(methyl methacrylate) bone cement. Flexural strength and bacterial viability were used to evaluate the modified cements. Effects of substitute chain length, polymer molar ratio and polymer weight ratio were studied. Results showed the modified cement exhibited a significantly enhanced antibacterial activity along with decreased flexural strength and flexural modulus. It was found that increasing the substitute chain length, QAB molar ratio and copolymer weight ratio significantly increased antibacterial activity but meanwhile reduced flexural strength and modulus. The water sorption study found that short substitute chain length led to more water sorption than loner one.**

Key words*: *antibacterial; bone cement; bacterial viability; flexural strength

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I. INTRODUCTION

Polymethyl methacrylate (PMMA) bone cement has been used in artificial joints surgery, especially total hip replacement surgery, for prosthesis fixation for over sixty years [1-3]. So far this cement is still a major product in orthopedic applications, due to its biocompatibility and satisfied regulatory requirement. Due to post-surgical infection, antibiotics have been added into bone cements [3]. It is known that post-surgical infection by bacteria is one of the most serious complications in orthopedic surgery for implantation [4, 5]. The infection is reported not only to prolong the hospitalization but also to cause revision surgery which in some cases could lead to severe emotional and physical pain to a patient. That is why most current commercially available bone cements contain antibiotics in their formulations [3, 5-8]. In addition to antibiotics, silver particles or silver nanoparticles and gold nanoparticles were also reported to show strong antibacterial functions after incorporation to PMMA bone cements [9-11]. The mechanism for using either antibiotics or silver or gold particles to inhibit or kill bacteria is based on the principle of drug release or slow-release. However, release or slow-release of drugs can suffer from a mechanical property reduction of the cement over time [6], short-term effectiveness but long-term run-out of drugs [7], an enhanced risk of bacteria developing antibiotic-resistance due to decreasing concentration of drugs [8], and potential cytotoxicity from drugs [9]. Recently, another different antibacterial approach was proposed and used in applications, that is, “kill by contact” [12]. This strategy was conducted by covalently link antibacterial drugs or compounds onto polymers, restoratives, or medical devices [12-25], which was believed to be more effective strategy as compared to release or slow release. The most commonly studied compounds are quaternary ammonium salts and their derivatives [12-25]. Theses compounds were derivatized and covalently linked to restoratives or medical devices [12-25]. The examples of using such compounds for medical applications include incorporating quaternary ammonium polyethylene nanoparticles into PMMA bone cement [13], adding quaternarized chitosan nanoparticles into PMMA bone cement [14], and using imidazolium-containing mono-methacrylates to copolymerize with MMA *in situ* [15]. applying polymerizable methacryloyloxydodecyl pyridinium bromide in resin composites [18], using curable methacryloxylethyl cetyl ammonium chloride in antibacterial bonding agents [20], adding polyethylenimine quaternary ammonium nanoparticles to resin composites [21], and incorporating polymerizable quaternary ammonium bromide derivatives with different chain lengths into glass-ionomer cements [22-24]. The results showed that all the quaternary ammonium salt-modified restoratives or cements exhibited significant antibacterial activities. This type of antibacterial materials was also found to demonstrate a broad spectrum of antimicrobials and to be able to kill or inhibit bacteria that have resistance to other types of cationic antibacterial compounds [25]. Our research group has been working with QAS for improved antibacterial dental restoratives for many years [22-24]. To extend our research to orthopedic applications, in this study we proposed to synthesize an antibacterial copolymer containing both QAS and MMA to partially replace PMMA in current PMMA bone cement for preventing potential post-surgical infection.

The purpose of this study was to synthesize antibacterial copolymers composed of both QAS and MMA, use the polymer to partially replace PMMA in

current PMMA bone cement, and evaluate flexural strength and antibacterial activity of the formed bone cements.

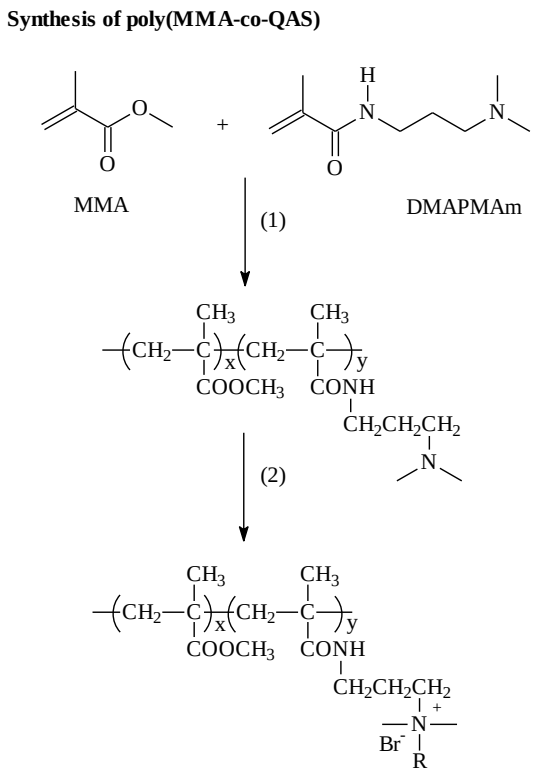
II. Material and Methods

A. Materials

Dimethylaminopropyl acrylamide (DMAPMAm), bromoethane, bromobutane, bromohexane, bromodecane, bromdodecane, 2,2'-azobisisobutyronitrile, ethanol, hexane, 2-hydroxyethyl methacrylate, toluene, ethanol and methyl methacrylate were received from Sigma-Aldrich Co. (Milwaukee, WI) and used without further purifications. Commercial PMMA bone cement was used as received form Wright Medical Technologies (Arlington, TN).

B. Synthesis and Characterization

Synthesis of poly(MMA-co-DMAPMAm): To a solution containing MMA and DMAPMAm in ethanol, a solution of 2,2'-azobisisobutyronitrile in ethanol was added. The polymerization reaction was carried out under nitrogen blanket at 65 ^{o}C for 24 h, followed by precipitating with hexane to obtain a solid product (yield > 95%). The molar feed ratio (MMA:DMAPMAm = 70:30 by mole) was used. The scheme for copolymer synthesis is described in Fig. 1A.



where (1) AIBN/65 ^{o}C/24 h; (2) RBr, 40 ^{o}C/24 h. Polymer molar ratio x : y = 75:25, 50/50, 25/75, or 80/20. R = -CH_2CH_3, -$(CH_2)_3CH_3$,-$(CH_2)_5CH_3$, -$(CH_2)_9CH_3$ and -$(CH_2)_{11}CH_3$.

Fig. 1 Schematic diagrams for synthesis of MMA and quaternary ammonium bromide copolymers

Synthesis of poly(MMA-co-DMAPMAm) with quaternary ammonium bromide: To a solution containing *poly(MMA-co-DMAPMAm)* in ethanol, bromoethane was added. The reaction was run at 40 ^{o}C for 24 h, followed by precipitating with hexane to obtain a solid product. Alkyl bromides with different chain length were prepared similarly. The synthesis scheme is shown in Fig. 1B.

Characterization: The synthesized antibacterial copolymers were characterized with Fourier transform-infrared (FT-IR) spectrometry and differential scanning calorimetry (DSC). FT-IR spectra were acquired on a FT-IR spectrometer (Mattson Research Series FT/IR 1000, Madison, WI). The DSC thermal history of selected antibacterial copolymers was determined on a DSC calorimeter (Perkin Elmer DSC, Shelton, CT) at a heating rate of 10°C/min under nitrogen.

C. Evaluation

Specimen preparation for evaluations: The formulations of the experimental antibacterial cements were prepared with a powder-liquid system [26]. The liquid MMA was used as received. The PMMA powder was mixed with the newly synthesized antibacterial poly(MMA-co-DMAPMAm-QAS) copolymer using a vortex mixer at the ratios or percentage described in the results. A powder/liquid ratio of 2/1 (wt/wt) was applied throughout the study unless specified. PMMA bone cement was used as control.

Specimens were prepared by mixing the MMA liquid with the polymer powder thoroughly at room temperature per manufacturer's instruction. Briefly, the rectangular specimens were made in a split Teflon mold with dimensions 3 mm (width) x 3 mm (thickness) x 25 mm (length) for flexural strength (FS) test. The cylindrical specimens were made in glass tubing with dimensions of 4 mm (diameter) x 2 mm (depth) for antibacterial tests. After 5-10 min, the cured specimens were removed from the molds and conditioned in distill water at 37 ^{o}C for 24 h before testing.

Flexural strength test: Flexural strength test was performed on a screw-driven mechanical tester (QTest QT/10, MTS Systems Corp., Eden Prairie, MN), with a crosshead speed of 1 mm/min [27]. A three-point bending fixture with a span of 20 mm between supports was used to conduct the flexural strength test. Six specimens were tested to obtain a mean value for each material or formulation in each test. Flexural strength was obtained from the equation $3Pl/2bd^2$, 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. Flexural modulus was obtained from the stress-strain curves of the flexural strength test.

Bacterial viability test: The bacterial viability test was carried out based on the protocol elsewhere [28]. In short, bacterial colonies were suspended in 5 mL of

tryptic soy broth, supplemented with 1% sucrose, to form a suspension with 10^8 CFU/mL of bacteria and incubated for 24 h. Three bacteria species including *S. mutans, S. aureus* and *P. aeruginosa* were assessed. The disk specimen was sterilized with 70% ethanol for 10 s and incubated with the bacterial suspension in tryptic soy broth at 37°C for 48 h under 5% CO_2. To 1 mL of the above bacterial suspension, 3 µL of a green/red (1:1 v/v) dye mixture (LIVE/DEAD BacLight bacterial viability kit L7007, Molecular Probes, Inc., Eugene, OR, USA) was added, followed by vortexing for 10 s, sonicating for 10 s, vortexing for another 10 s and keeping in dark for about 15 min before analysis. Then 20 µL of the stained bacterial suspension was added onto a glass slide and viable bacteria (green) were imaged with an inverted fluorescence microscope (EVOS FL, AMG, Mill Creek, WA, USA). A bacteria suspension without disks was used as control and viable bacteria counts form the suspension were used as 100%. The viability was analyzed by counting from the recorded images. Triplicate samples were used to obtain a mean value for each material.

Water sorption test: Water sorption and solubility were determined according to ISO 4049 [29]. Briefly, disc specimen (15 mm in diameter x 1 mm in thickness) of the resin composite was prepared in a metal ring mold with glass slides covered on both sides, followed by photo-curing with blue light for 2 min on each side. Upon removal, the specimen was placed in a desiccator and maintained at 37 ^oC until its weight was constant. The weight was recorded as m_0. Then the specimen was immersed in distilled water at 37 ^oC for 1, 3 and 7 days, followed by removing from water, blotting dry and weighing. The weight was recorded as m_1. Water sorption (WS) was calculated according to the equation $(m_1 – m_0)/V$, where V = volume of the disc specimen. Then the same specimen was reconditioned in the desiccator and maintained at 37 ^oC until its weight was constant. The weight was recorded as m_1. The mean value was averaged from three readings (n = 3).

Statistical analysis: One-way analysis of variance (ANOVA) with the post hoc Tukey-Kramer multiple-range test was used to determine significant differences of the measured properties among the materials or formulations in each group. A level of $\alpha = 0.05$ was used for statistical significance.

III. Results and Discussion

A. Characterization

Fig. 2 shows two sets of FT-IR spectra in absorbance for poly(MMA-co-QAS) synthesis: (A) change of molar ratio and (B) change of chain length on QAS. In Fig. 2A, Spectrum a represents PMMA, which shows two peaks at 3200 and 3070 for methyl group and 1730 for ester group. Spectra b, c, d, e and f represent the peaks for poly(MMA-co-QAS) at the molar ratios of 90/10, 80/20, 70/30, 60/40 and 50/50, respectively. Spectrum a shows the peaks at 3625 for dimethyl propylacrylamide, 3370 and 3250 for QAS group, 3080-2800 for methyl and methylene groups, 1730 for ester group, and 1680 for amide group. Apparently, increasing QAS molar ratio from 10% to 50% from Spectra b to f decreased the peak intensity at 3650 for dimethyl propylacrylamide but increased the peak intensities at 3370 and 3250 for QAS, 3080-2800 for methyl and methylene groups and 1680 for amide from acrylamide. The result indicates that QAS was successfully grafted onto poly(MMA-co-DMAPMAm). In Fig. 2B, Spectra a, b, c and d represent a set of spectra with different chain lengths grafted onto poly(MMA-co-DMAPMAm) copolymer. Apparently, increasing chain length from PMMA (a), BrC2 (b), BrC6 (c) and BrC12 (d) significantly increased the peak intensities at 3370 and 3250 for QAS, 3080-2800 for methyl and methylene groups and 1680 for amide from acrylamide, which is similar to those shown in Fig. 2A. The result also indicates that QAS with different chain lengths was successfully grafted onto poly(MMA-co-DMAPMAm).

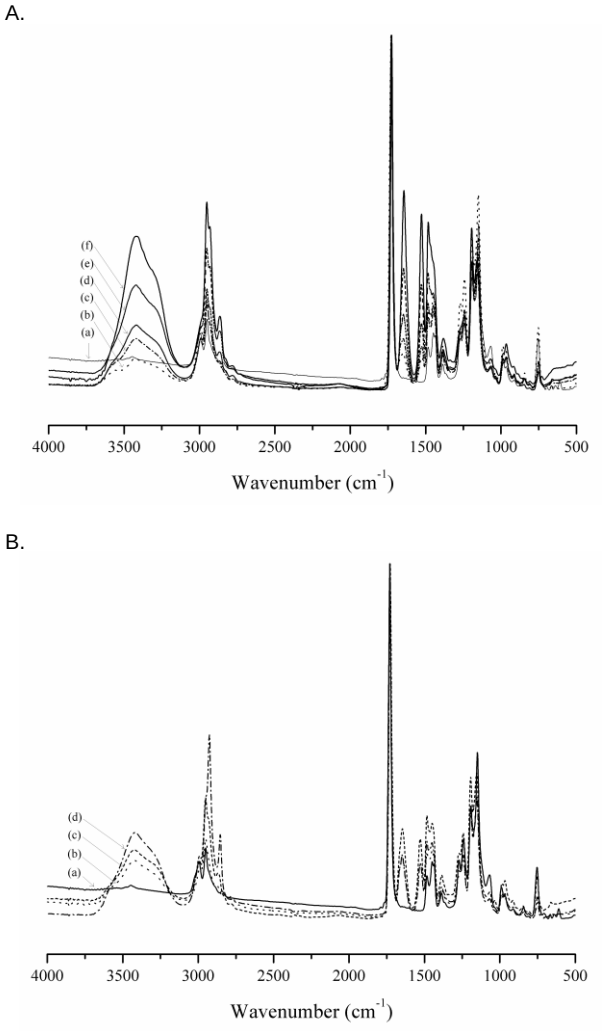


Fig. 2 FT-IR spectra of a set of copolymers with peak changes: A: (a) PMMA, (b) 90/10, (c) 80/20, (d) 70/30, (e) 60/40 and (f) 50/50; B: (a) PMMA, (b) BrC2, (c) BrC6 and (d) BrC12.

B. Evaluation

Fig. 3 shows the effect of chain length on FS and *S. aureus* viability. The mean FS (MPa) values was in the decreasing order of PMMA > BrC2 > BrC4 = BrC6 > BrC10 = BrC12, where there were no significant differences between BrC4 and Br C6 and between BrC10 and BRC12 ($p > 0.05$). The mean *S. aureus* viability was in the decreasing order of PMMA > BrC2 > BrC4 > BrC6 > BrC10 > BrC12. Apparently, all the QAS-modified cement showed significantly lower FS as compared to PMMA cement. Increasing chain length decreased FS. On the other hand, increasing chain length significantly reduced bacterial viability or in other words increased antibacterial activity. The significant decrease in FS may partially be attributed to water sorption by QAS. The QAB by nature is a quaternary ammonium salt (QAS) carrying both positive and negative charges, which absorb water [30]. Since water serves as a plasticizer in the material [31], the QAS-containing material behaves like a hygrogel more or less [32]. The reduced FS may also partially be attributed to the reason that bulky QAS moiety increases the free space between polymer chains [33], which leads to polymer entanglement reduction and thus mechanical strength reduction [34, 35]. The antibacterial result was very consistent with those reports elsewhere [36].

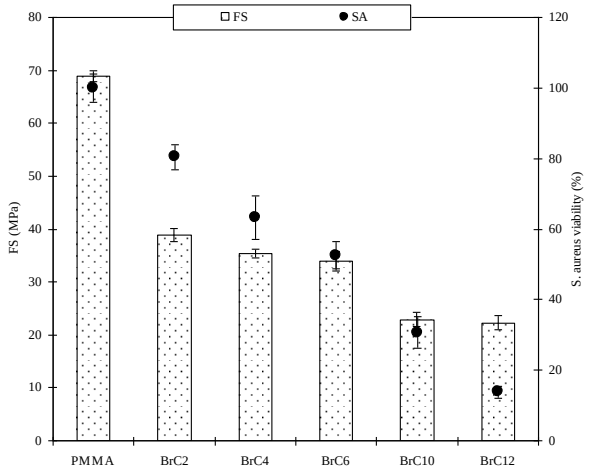


Fig. 3 Effect of chain length on FS and S. aureus viability: MMA/QAS = 70/30 by mole in P(MMA-co-QAS); PMMA:P(MMA-co-QAS) 50/50 by weight; P/L =2/1 by WEIGHT

Fig. 4 shows the effect of molar ratio on FS and *S. aureus* viability. The mean FS (MPa) value was in the decreasing order of PMMA > 90/10, 80/20, 70/30, 60/40 and 50/50 ($P < 0.05$). The mean *S. aureus* viability was in the decreasing order of PMMA > 90/10, 80/20, 70/30, 60/40 and 50/50. Similar to the result shown in Fig. 3, increasing the molar ratio or increasing the molar ratio of QAS in copolymer decreased both FS and bacterial viability. For FS, increasing QAS content increases the free space between polymer chains, thus decreases FS. Increasing antibacterial QAS content increased antibacterial component and that is why bacterial viability decreased. The molar ratio at 50/50 showed a 90% reduction in bacterial viability.

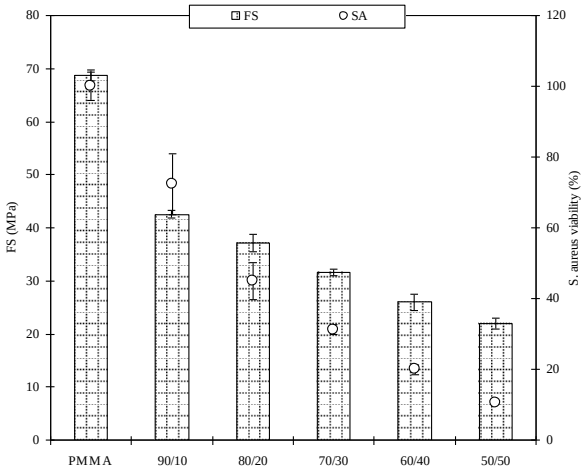


Fig. 4 Effect of molar ratio on FS and S. aureus viability: PMMA:P(MMA-co-QAS) 50/50 by weight, P/L =2/1 by weight

Fig. 5 shows the effects of antibacterial QAS moieties on water sorption of the formed PMMA cement. Apparently both QASBrC6 and QASBrC12 modified copolymers showed higher water sorption as compared to PMMA cement. At Day 1, PMMA, QASBrC6 and QASBrC12 showed 0.7%, 15% and 13% increase in water sorption, respectively. At Day 3, PMMA, QASBrC6 and QASBrC12 showed 1.2%, 0.3% and 0.3% increase in water sorption. At Day 7, PMMA, QASBrC6 and QASBrC12 showed 0.9%, 0.6% and 0.5% increase in water sorption. Apparently, at Day 7, the water sorption for each material reached hyperbolic or in other words, no more water would be absorbed by each material. In comparison with PMMA, QASBrC6 and QASBrC12, QASBrC6 showed 21, 0.25, and 0.67 times more water sorption than PMMA at Day 1, 3 and 7, respectively. QASBrC12 showed 18, 0.25 and 0.55 times more water sorption than PMMA at Day 1, 3 and 7, respectively. QASBrC12 showed slightly less water sorption than QASBrC6. PMMA is a very hydrophobic polymer. QAS-containing polymers are known to absorb water [30]. That is why poly(MMA-co-DMAPMAm-QAS) showed significant water sorption characteristics. The reason why QASBrC6 showed more water sorption than QASBrC12 is may be attributed to that QASBrC12 contains a longer hydrophobic chain (C12) than QASBrC6, leading to more hydrophobic in QASBrC12-composed PMMA cement than OASBrC6-composed one. Further, considering one mole of poly(MMA-co-DMAPMAm-QAS), the one with QASBrC12 should contain less hydrophilic QAS as compared to the one with QASBrC6. That may be why the QASBrC6-composed cement showed higher water sorption than the QASBrC12-composed cement.

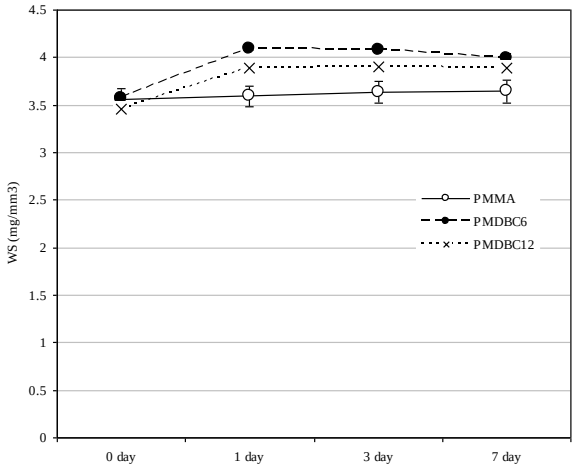


Fig. 5 Water sorption of the tested cements: MMA/QAS = 25/75 by mole in P(MMA-co-QAS); PMMA:P(MMA-co-QAS) 50/50 by weight, P/L =2/1 by weight

Table 1 shows the effect of poly(MMA-co-QAB) copolymers on the viability of three bacterial species, *S. aureus*, *E. coli* and *P. aeruginosa.* For chain length, increasing chain length significantly decreased the viability of all three bacteria. From BrC2 to BrC12, *S. aureus*, *E. coli* and *P. aeruginosa* showed 86%, 95% and 92% decrease in viability, respectively, indicating that the response of different bacteria species to QAB is slightly different. The result also indicates that the QAB-containing experimental PMMA cement has a broad spectrum of antibacterial properties. For molar ratio, *S. aureus*, *E. coli* and *P. aeruginosa* showed 90%, 94% and 94% decrease in viability, respectively, indicating that more QAB on the copolymer the stronger antibacterial activity. For weight ratio (a ratio of poly(MMA-co-QAB)/PMMA), *S. aureus*, *E. coli* and *P. aeruginosa* showed 90%, 94% and 97% decrease in viability, respectively, indicating that increasing the content of the synthesized QAB-containing copolymer in entire PMMA powder will increase antibacterial function with a long chain BrC12 showing the stronger impact. Table 2 shows the effect of poly(MMA-co-QAB) copolymers on FS and FM. For the effects of chain length, molar ratio and weight ratio, increasing these parameters significantly decreased both FS and FM, with no exception. The results can be attributed to increased free space between polymer chains and mainly to absorbed water for plasticizing effect.

TABLE I. EFFECT OF P(MMA-CO-QAB) COPOLYMERS ON *S. AUREUS*, *E. COLI* AND *P. AERUGINOSA* VIABILITY

Effect of chain length

	PMMA	BrC2	BrC4	BrC6	BrC10	BrC12
S. A.	100 (4.1)	80.4 (3.5)	63.3 (6.3)	52.5 (3.8)	30.7 (4.5)	13.7 (1.8)
E. C.	100 (6.1)	60.5 (9.7)	38.3 (1.5)	21.3 (2.9)	15.5 (2.6)	4.9 (0.5)
P. A.	100 (4.1)	42.3 (6.3)	37.7 (3.9)	24.8 (2.3)	19.8 (1.7)	7.9 (1.6)

Effect of molar ratio

	PMMA	90/10	80/20	70/30	60/40	50/50
S. A.	100 (4.1)	72.2 (8.6)	45.0 (5.3)	31.2 (1.4)	19.9 (1.4)	10.4 (0.4)
E. C.	100 (6.1)	89.9 (1.9)	30.3 (3.7)	17.9 (2.9)	7.1 (0.6)	6.3 (0.7)
P. A.	100 (4.1)	39.1 (3.5)	16.5 (1.8)	14.7 (1.2)	7.0 (0.6)	5.7 (1.1)

Effect of weight ratio

	PMMA	7525 BC6	5050 BC6	2575 BC6	7525B C12	5050B C12	2575 BC12
S. A.	100 (4.1)	61.0 (9.1)	29.7 (0.9)	14.3 (1.8)	37.3 (0.5)	26.9 (1.4)	10.1 (1.1)
E. C.	100 (6.1)	28.8 (1.3)	19.1 (1.4)	8.0 (0.9)	27.3 (3.9)	14.7 (0.3)	5.9 (0.3)
P. A.	100 (4.1)	18.9 (0.8)	16.1 (1.1)	7.8 (1.5)	28.2 (7.2)	14.6 (2.5)	3.2 (0.6)

*Specimens were cultured with *bacteria* for 48 h before testing. S.A., E.C., and P.A. represent *S. aureus, E. coli and P. aeruginosa*, respectively. Entries are mean values with standard deviations in parentheses and the mean values with the same superscript letter were not significantly different ($p > 0.05$).

TABLE II. EFFECT OF P(MMA-CO-QAB) COPOLYMERS ON FM (GPA) AND FS (MPA)

Effect of chain length

	PMMA	BrC2	BrC4	BrC6	BrC10	BrC12
FM	2.29 (0.04)	1.74 (0.06)	1.57 (0.05)	1.42 (0.17)	1.24 (0.03)	0.82 (0.06)
FS	68.8 (1.0)	38.8 (1.2)	35.5 (0.8)	33.9 (1.8)	22.9 (1.3)	22.3 (1.3)

Effect of molar ratio

	PMMA	90/10	80/20	70/30	60/40	50/50
FM	2.29 (0.04)	2.06 (0.11)	2.01 (0.13)	1.34 (0.04)	0.98 (0.06)	0.92 (0.12)
FS	68.8 (1.0)	42.5 (0.7)	37.1 (1.7)	31.5 (0.7)	26.0 (1.5)	21.9 (1.1)

Effect of weight ratio

	PMMA	7525B C6	5050B C6	2575B C6	7525B C12	5050B C12	2575 BC12
FM	2.29 (0.04)	2.39 (0.09)	1.71 (0.12)	0.44 (0.02)	2.03 (0.13)	1.42 (0.08)	0.66 (0.03)
FS	68.8 (1.0)	49.1 (2.0)	32.2 (2.0)	13.0 (0.3)	43.2 (1.7)	27.7 (0.7)	18.4 (2.1)

*Specimens were conditioned in distilled water at 37 °C for 24 h before testing. Entries are mean values with standard deviations in parentheses and the mean values with the same superscript letter were not significantly different ($p > 0.05$).

IV. Conclusions

We have synthesized a novel QAS-composed PMMA copolymer and incorporated it into a traditional PMMA bone cement formulation. The modified cement showed a significantly enhanced antibacterial activity along with decreased flexural strength and flexural modulus. It was found that increasing the substitute chain length, QAB molar ratio and copolymer weight ratio significantly increased antibacterial activity but meanwhile reduced flexural strength and modulus. The water sorption study found that short substitute chain length led to more water sorption than longer one.

Acknowledgment

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