Journal of Multidisciplinary Engineering Science and Technology (JMEST)
ISSN: 2458-9403
Vol. 3 Issue 12, December - 2016

Preparation of An Experimental Light-cured Antibacterial Dental Cement

Yuling Xu[1,2], Leah Howard[2], Dong Xie[2*]

1- College of Chemical and Environmental Engineering, Wuhan Polytech University, Wuhan, China
2- Department of Biomedical Engineering, Indiana University-Purdue University Indianapolis, Indianapolis, USA

(e-mail: dxie88@yahoo.com)

***Abstract*—An experimental lightcured antibacterial dental glass-ionomer cement containing furaneol has been developed. Compressive strength (CS) and S. mutans viability were used to evaluate the mechanical strength and antibacterial activity of the formed cement. Yield strength (YS), modulus (M), diametral tensile strength (DTS) and flexural strength (FS) were also determined. The formulated antibacterial cements showed a significant antibacterial activity, accompanying with an initial CS reduction. The effects of furaneol content, GM grafting ratio, P/W ratio, P/L ratio, human saliva, and aging in water on CS and S. mutans viability were evaluated. The results showed that increasing the furaneol content increased the antibacterial activity but decreased CS. Increasing the GM grafting ratio increased CS. Increasing P/W ratio increased both CS and antibacterial activity. Increasing P/L ratio increased CS but decreased the antibacterial activity. The antibacterial cement also showed a similar antibacterial activity to lactobacillus, S. aureus and S. epidermidis. The human saliva did not affect the antibacterial activity of the cement. The result also indicates that the antibacterial cement may have a long-lasting antibacterial function.**

Keywords—antibacterial polymer; S. mutans viability; glass-ionomer, compressive strength

I. INTRODUCTION

Recently bacteria invasion and infection have attracted more and more attentions [1]. In dentistry, bacteria invasion has been mainly related to tooth caries and periodontal diseases [2]. There are two major types of dental caries, primary and secondary. Primary caries is often referred to those caries formed naturally due to released acids and sugars from food, beverages and fruits, whereas secondary caries is defined as those produced after dental restorations [3]. Secondary caries is found to be the main reason to the restoration failure of dental restoratives including resin composites and glass-ionomer cements [4-7]. Secondary caries often occurs at the interface between the restoration and the cavity preparation. One of the main reasons to cause secondary caries is demineralization of tooth structure due to invasion of plaque bacteria (acid-producing bacteria) such as *Streptococcus mutans* (*S. mutans*) and *lactobacilli* in the presence of fermentable carbohydrates [7]. Although numerous efforts were 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 [8-12]. Yet release or slow-release can lead or has led to a reduction of mechanical properties of the restoratives over time, short-term effectiveness, and possible toxicity to surrounding tissues if the dose or release is not properly controlled [8-12]. Materials containing quaternary ammonium salt (QAS) or phosphonium salt groups have been studied extensively as an important antimicrobial material and used for a variety of applications due to their potent antimicrobial activities [13, 14]. These materials are found to be capable of reducing the number of bacteria that are resistant to other types of cationic antibacterials [15]. The examples of the QAS-containing materials as antibacterials for dental restoratives include incorporation of a methacryloyloxydodecyl pyridinium bromide as an antibacterial monomer into resin composites [14], use of methacryloxylethyl cetyl ammonium chloride as a component for antibacterial bonding agents [16, 17], addition of quaternary ammonium polyethylenimine nanoparticles into composite resins [18], and incorporation of polyQAS (PQAS) into glass-ionomer cements (GICs) [19]. All these studies found that the QAS-containing materials did exhibit significant antibacterial activities. However, it has been reported that human saliva can significantly reduce the antibacterial activity of the QAS-containing restoratives, probably due to electrostatic interactions between QAS and proteins in saliva [20, 21]. Recently furanone derivatives have been found to have strong antitumor [22] and antibacterial functions [23]. These compounds all contain a furanone structure (classified as lactone or butenolide) and showed a similarity to natural manoalide, which has an interesting anti-inflammatory activity [24]. The similar compounds were also found to show an inhibitory effect on bacterial quorum-sensing [25], probably due to the structural similarity to autoinducers in bacteria. The exact antibacterial mechanism is still unclear and under investigation. Furaneol, extracting from many fruits, was also found to show significant antimicrobial functions to both bacteria and fungi [26] without

hemolytic activity to human. In this study, we hypothesize to derivatize this natural compound and incorporate it into dental GICs, which may allow us to explore it in dental applications.

The objective of this study was to derivatize furaneol, incorporate it onto polymer backbone, formulate the resultant light-curable glass-ionomer cements, and study the effect of this derivative on the compressive strength and antibacterial activity of the formed cements. DTS and FS were also determined.

II. Material and Methods

A. Materials

Furaneol (F), N,N'-dicyclohexylcarbo diimide (DCC), pyridine, acrylic acid (AA), itaconic acid (IA), 2,2'-azobisisobutyronitrile (AIBN), dl-camphoroquinone (CQ), 2-(dimethylamino)ethyl methacrylate (DMAEMA), pyridine, glycidyl methacrylate (GM), tetrahydrofuran (THF), ether and N,N-dimethylformamide (DMF) were used as received from Fisher Scientific (Waltham, MA) without further purifications. Commercial glass-ionomer cements Fuji II, Fuji II LC and their corresponding glass powders were used as received from GC America Inc (Alsip, IL).

B. Synthesis and Characyterization

Three synthesis steps have been used, i.e., synthesis of the star-shaped polymer, grafting F onto the polymer and grating GM onto the polymer. (1) A 4-arm star-shaped chain-transfer agent (CTA) was prepared as described elsewhere [27]. The star-shaped poly(AA-co-IA) copolymer was synthesized via free-radical polymerization. Briefly, to a flask containing AA (36 mmol), IA (9 mmol), 4-arm CTA (0.25 mmol) and distilled water (40 ml), AIBN (0.05 mmol) was added. After degassing and nitrogen-purging, the solution was heated to 70 ^{o}C and kept at that temperature for 17 h. The molar feed ratio (AA:IA = 7:3 by mole) was used as suggested elsewhere [28]. The formed polymer (yield = 96%) was freeze-dried and stored prior to use. (2) To a solution containing poly(AA-co-IA) polymer in THF, F, pyridine (1% by weight) and DCC (equivalent to F in mole) was added. The ratio of carboxylic acid verse F by mole was varied (95/5, 93/7, 90/10, 85/15 and 80/20). The reaction was run at room temperature overnight. Then the precipitates dicyclohexylurea (DCU) were filtered and the F-containing poly(AA-co-IA) copolymers or PAIF were purified by precipitation from ether. The synthesis scheme is also shown in Fig. 1. (3) The F-containing star-shaped poly(AA-co-IA) or PAIF with pendent methacrylate groups was synthesized similarly as described elsewhere [19]. Briefly, the polymer PAIF was tethered with GM in DMF at 50 ^{o}C overnight in the presence of pyridine (1% by weight). The GM-tethered polymer or PAIFG was then recovered by precipitation from diethyl ether, followed by drying in vacuo at room temperature. The synthesis scheme is also shown in Fig. 1.

The chemical structures of the synthesized polymer and its precursors were characterized by Fourier transform-infrared (FT-IR) spectroscopy. The formed polymer was characterized by gel permeation chromatography (GPC). FT-IR spectra were obtained on a FT-IR spectrometer (Mattson Research Series FT/IR 1000, Madison, WI). For determination of molecular weight, the polymer was treated with diazomethane, which was generated from diazald reacted with potassium hydroxide (KOH) in water/ethanol solution at 65 ^{o}C, to obtain partially esterified products [29], having solubility in THF for molecular weight estimation. Molecular weight was estimated on a Waters GPC unit (Model 410 differential refractometer, Waters Inc., Milford, MA) with THF as a solvent, using standard GPC techniques and polystyrene standards.

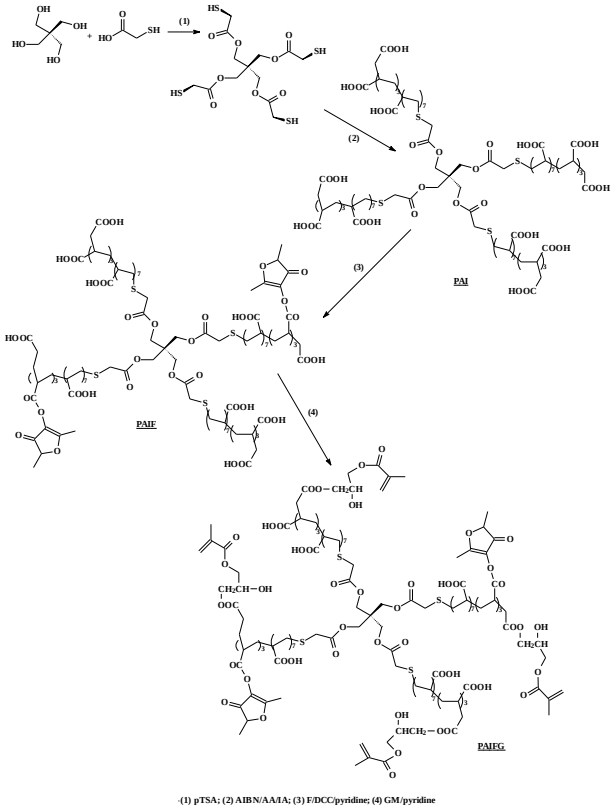

Fig. 1. Schematic diagram for synthesis of the star-shaped poly(AA-co-IA) with pendent F and methacrylate.

C. Evaluation

The experimental cements were formulated with a two-component system (liquid and powder) [29]. The liquid was formulated with the light-curable antibacterial polymer PAIFG, water, 0.9% CQ (photo-initiator, by weight) and 1.8% DMAEMA (activator). The polymer/water (P/W) ratio = 60:40 (by weight). The glass powder was Fuji II LC glass powder and the powder/liquid (P/L) ratio = 2.7 (by weight).

Specimens were fabricated at room temperature according to the published protocol [29]. 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 (LED, 30W, EXAKT 520 Blue Light Polymerization Unit, EXAKT Technologies, Inc., Oklahoma City, OK) for 2 min, followed by conditioning in 100% humidity at room temperature for 15 min, removing from the mold and conditioning in distilled water at 37 ^{o}C for 24 h prior to testing, unless specified.

CS, DTS and FS tests were performed on a screw-driven mechanical tester (QTest QT/10, MTS Systems Corp., Eden Prairie, MN), with a crosshead speed of 1 mm/min. The FS test was performed in three-point bending with a span of 20 mm between supports. Six 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. Compressive yield strength (YS) and modulus (M) were obtained from the stress-strain curves of CS tests.

The antibacterial test was conducted following the published procedures [19]. *S. mutans* was mainly used to evaluate the antibacterial activity of the studied cements throughout the study. Other bacteria including lactobacillus, S. aureus and S. epidermidis were also used to evaluate a broad 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. Each cement specimen was dipped in 70% ethanol for 10 sec, followed by drying in the air for another 10-20 sec and placing in a vial containing 5 ml TSB supplemented with 1% sucrose. To the specimen-containing TSB, 100 µl of the above incubated S. mutans suspension was added. After incubating at 37 ^{o}C for 48 h under anaerobic condition with 5% CO_2, the specimen-containing suspension was sonicated for 20 sec to remove the adhered bacteria off the specimen. 1 ml of the suspension was then used to mix with 3 µl of a two-color dye, which was formed by thoroughly mixing equal volumes of the red and the green dyes (LIVE/DEAD BacLight bacterial viability kit L7007, Molecular Probes, Inc., Eugene, OR, USA) in a microfuge tube for 1 min. The formed mixture was vortexed for 10 sec, sonicated for 10 sec, vortexed 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, USA). Triple replica was used to obtain a mean value for each material.

Human saliva, obtained from a healthy volunteer, was centrifuged for 15 min at 12,000g to remove debris [20]. After the supernatant was filtered with a 0.45 µm sterile filter, the filtrate was stored in a -20 ^{o}C freezer prior to use. The sterilized cement specimen (see 2.3.4) was incubated in a small tube containing 1 ml of saliva at 37 ^{o}C for 2 h, followed by placing in 5 ml TSB supplemented with 1% sucrose. The rest of the procedures for antibacterial test are the same as described above.

The specimens for both CS and antibacterial activity aging tests were conditioned in distilled water at 37 ^{o}C for 1, 3, 7, 14 and 30 days, followed by direct testing for CS (see above for details) and incubating with S. mutans for 48 h for antibacterial testing (see above for details).

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 or formulations in each group. A level of $\alpha = 0.05$ was used for statistical significance.

III. Results and Discussion

A. Characterization

Table I shows the FT-IR spectra for F, GM, PAI, PAIF and PAIFG. Disappearance of the broad peak (cm^{-1}) at 3251 for hydroxyl group on F, formation of a broader and wider peak between 3500 and 2750 for COOH, formation of a wider and stronger peak at 1729 for carbonyl groups on both PAI and F, and appearance of a new peak at 1604 for C=C group on F confirmed successful attachment of F onto PAI. By comparing the spectra for GM, PAIF and PAIFG, a longer peak on 1607 confirmed the successful addition of GM onto PAIF to form PAIFG. The molecular weight of the star-shaped poly(AA-co-IA) = 31,480 Daltons.

B. Evaluation

In preventive restorative dentistry, secondary caries is critical and prevention of secondary caries plays a key role in long-lasting restorations [4-7]. The antibacterial strategies include release or slow-release of various incorporated low molecular weight antibacterial agents such as antibiotics, zinc ions, silver ions, iodine and chlorhexidine [8-12] and mixing or attaching antibacterial agents such as quaternary ammonium salts (QAS) onto dental restoratives to kill bacteria by touch or simple contact [14-18]. Yet release or slow-release can lead or has led to a reduction of mechanical properties of the restoratives over time, short-term effectiveness, and possible toxicity to surrounding tissues if the dose or release is not properly controlled [8-12]. On the other hand, killing bacteria by touch or simple contact has recently

attracted a special attention [14-18]. The quaternary ammonium salts (QAS) and their functional derivatives have been studied extensively as an important antimicrobial material and used for a variety of applications due to their potent antimicrobial activities [14-18]. However, it has been reported that human saliva can significantly decrease the antibacterial activity of the QAS-containing restoratives, probably due to electrostatic interactions between QAS and proteins in saliva [20, 21]. Furanone-containing materials are reported to have a broad range of biological and physiological properties including antitumor, antibiotic, haemorrhagic and insecticidal activity [22], although the biological mechanism of these derivatives is still under investigation. F is a natural furanone analog, which exists in strawberry and other fruits. It has been reported that natural F not only kills G-positive but also destroys G-negative bacteria and even fungi [26]. To explore the antibacterial application of this natural compound in dental research, we incorporated it into dental GICs. The following discussion demonstrates how this compound was incorporated into our experimental GICs and its effect on the mechanical and antibacterial properties of the formed cements.

TABLE I. THE CHARACTERISTIC PAEKS FROM FT-IR SPECTRA

Code	The characteristic peaks from FT-IR
F	3251, 1308, 1151 and 759 (-OH), 2970, 2870, 1451, 1370 and 1002 (-CH and -CH_3), 1698 (carbonyl on -C=C-CO-), 1623, 1197 and 1042 (C=C on -C=C-O-C-), 1094 and 929 (ether on -C-O-C-)
GM	2957, 1453, 1378, 814 and 762 (-CH_3 and –CH), 1720, 1507 and 1403 (carbonyl), 1638, 1316 and 1017 (C=C), 1170 and 908 (-C-O-C-), 943, 843, 814 and 762 (-CH(O)-CH_2-)
PAI	2963 (2385-3687) (OH on –COOH), 2970, 2661, 1450, 1184 and 797 (-CH and CH_3), 1719 (carbonyl on -COOH)
PAIF	2937 (2385-3687) (OH on –COOH), 2661, 1450 and 1368 (-CH and -CH_3), 1729, 1509 and 1403 (carbonyl on both acrylate and –C=C-CO-), 1604 and 1196 and 1021 (C=C on -C=C-O-C-), 1262 and 939 (ether on -C-O-C-)
PAIFG	3400 (OH on GM), 2938 (2185-3673) (OH on –COOH), 2661, 1450, 1368, 813 and 744 (-CH and -CH_3), 1731, 1509 and 1403 (carbonyl on acrylate, methacrylate and –C=C-CO-), 1607 and 1194 and 1021 (C=C on -C=C-O-C-), 1262 and 963 (ether on -C-O-C-).

Fig. 2 shows the effects of F content on CS and S. mutans viability of the cements, respectively. Obviously with F addition the cement showed a reduction in both CS and S. mutans viability. The loss of CS can be attributed to the incorporated F because hydrophobic F did not contribute any strength enhancement to the cements. It is known that only pendent carboxyl groups and carbon-carbon double bonds in GIC cements contribute strength enhancement [30]. By comparing them with the cement without F (its CS = 236 MPa), the cements with 1%, 2% and 3% ratios showed a reduction of 8%, 15% and 23% in S. mutans viability but their CS were 233, 220, 196 MPa, respectively. The data indicate that the cement with 2% would be a better candidate for further evaluation because it showed a double cell viability reduction as compared to the one with 1% but still kept its CS close to commercial Fuji II LC (CS = 236.2 MPa, see Table III).

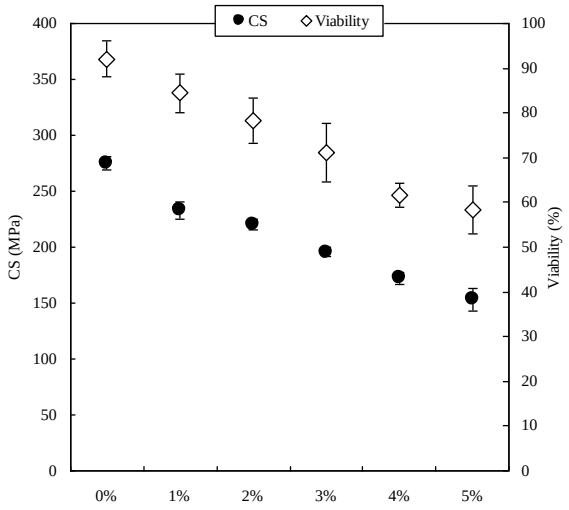

Fig. 2. Effect of F content on CS and S. mutans viability of the experimental cement.

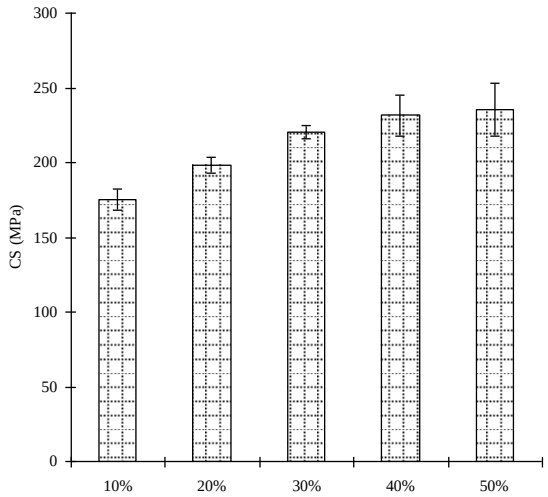

Fig. 3. Effect of GM-grafting ratio on CS of the experimental cement.

Fig. 3 shows the effect of GM grafting ratio on CS. CS was increased with increasing GM, where there were no significant differences between 30% and 40% and between 40% and 50% ($p > 0.05$). GM grafting from 10 to 30% significantly increased CS with an increase of 13 to 34%. Since the formulated cement is a light-curable cement and its strength partially depends on the carbon-carbon double bond (C=C) content on GM, it is necessary to investigate the effect of GM grafting ratio. The data in Fig. 3 indicate that increasing GM increased CS. The reason can be attributed to the fact that increasing GM increases the photo-curable C=C bond content thus increasing CS. Since GM was introduced by replacing some of the carboxyl groups on the polymer and had no effect on F, the bacterial viability testing was not necessary. During the study it was also found that when GM reached 50% the specimens were difficult to be fabricated. That is why the greater standard deviation value was observed. The experimental result showed that the cements with GM at 30%, 40% and 50% showed the CS values all above 200 MPa with statistically the same values, suggesting that GM over 30% may be used for further formulation and investigation.

Fig. 4 shows the effect of P/W ratio on CS and S. mutans viability. Increasing P/W ratio significantly increased CS with an increase of 15 to 51% but reduced cell viability with a reduction of 4 to 16%. In commercial glass-inonmer cements Fuji II LC and Vitremer, low MW comonomers such as HEMA, TEGDMA and other dimethacrylates are often incorporated to enhance the compatibility between the C=C bond-containing polymers and water due to enhanced hydrophobicity of the former [30]. However, these low MW molecules led to lower strength and cytotoxicity [31]. Our previous study showed that introducing GM could avoid using these low MW molecules because grafting one GM molecule could introduce one hydrophilic hydroxyl group [31, 32], thus leading to an enhancement in phase compatibility between polymers and water. Therefore only polymer and water were used to formulate the polymer liquid in this study. The effect of polymer/water (P/W) ratio was then evaluated. The more water the lower the mechanical strength [31]. Data in Fig. 4 showed that increasing P/W ratio increased CS but decreased the cell viability, which can be easily explained as the reason that increasing P/W ratio increases in situ curable carboxyl groups, light-curable C=C bonds, and antibacterial F in the cement. That is why both CS and antibacterial function were increased. The data also showed that 65/35 and 70/30 were statistically the same. In the study, we also found that 65/35 was easier to be mixed to form the cement than 70/30 (the latter was too viscous).

P/L ratio is another common parameter in formulating GICs. Either high or low P/L ratio does not favor mixing and mechanical strength [33]. In this study, P/L ratio also showed the effect on S. mutans viability. The data in Fig. 5 showed that increasing P/L ratio increased CS but reduced the antibacterial activity. The result is reasonable because a high P/L ratio indicates high glass powder but low polymer contents in the formulation. The higher P/L ratio the higher CS [33]. On the other hand, however, the lower polymer content in the formulation mean the lower F content in it. That is why increasing P/L ratio increased the cell viability or reduces the antibacterial activity. It was found that the P/L ratio at 2.7 showed the optimal CS and antibacterial activity. Both P/L at 3 and 3.2 showed higher bacterial viabilities, although their CS values were higher. In addition, during the study we found that both 3 and 3.2 were hard to be mixed due to their very high viscosities, which was also indicated from their high standard deviation values as shown in Fig. 5.

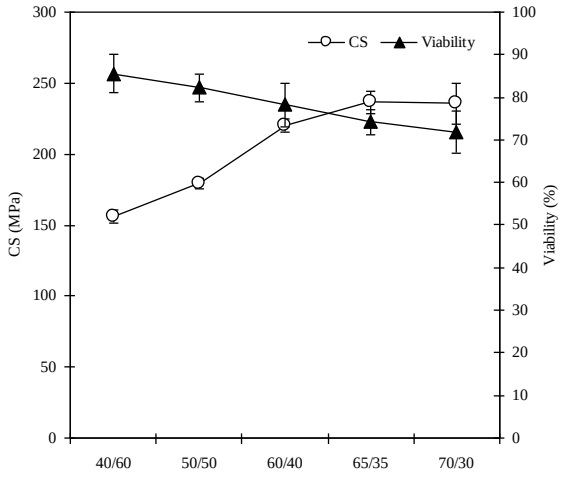

Fig. 4. Effect of P/W ratio on CS and S. mutans viability of the experimental cement

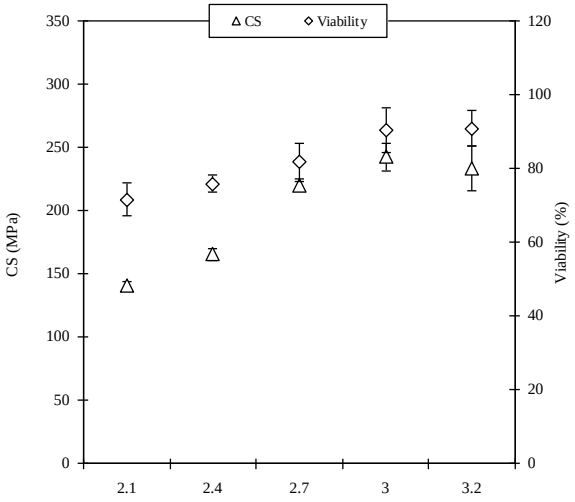

Fig. 5. Effect of P/L ratio on CS and S. mutans viability of the experimental cement.

Table II shows the effects of F on the viability of four bacteria including S. mutans, lactobacillus, S. aureus and S. epidermidis and human saliva on antibacterial cements. The cement with 2% F reduced

the viability of all four bacteria in the range of 56.3 to 81.4. Lactobacillus is another main oral cavity-producing bacterium although it is not as popular as S. mutans. S. aureus and S. epidermidis are two major bacteria that often cause skin and implant infections. To examine the antibacterial activity of F on these bacteria, we compared the viability of all the four bacteria after incubating with the cement specimens, among which lactobacillus showed the lowest viability (56.3%), followed by S. mutans, S. epidermidis and S. aureus.

Table II also shows that human saliva nearly exerts no effect on the S. mutans viability after culturing with the antibacterial cements. No statistically significant differences in the S. mutans viability were found between the cements with and without human saliva treatment. It has been noticed that saliva can significantly reduce the antibacterial activity of the QAS or PQAS-containing materials based on the mechanism of contact inhibition [20, 21]. The reduction was attributed to the interaction between positive charges on QAS or PQAS and amphiphilic protein macromolecules in saliva, thus leading to formation of a protein coating which covers the antibacterial sites on QAS or PQAS [20, 21]. Unlike QAS or PQAS, F does not carry any charges. That may be why the F-containing cement did not show any reduction in antibacterial activity after treating with saliva.

TABLE II. EFFECT OF F ON DIFFERENT BACTERIA VIABILITY AND HUMAN SALIVA ON ANTIBACTERIAL CEMENT

S.M.	LAC	S.A.	S.E
Cements without saliva treatment			
74.2 (2.9)[a]	56.3 (3.4)[b]	81.4 (3.5)[c]	79.3 (5.1)[d]
Cements with saliva treatment			
72.9 (5.6)[a]	59.2 (6.3)[b]	83.5 (4.1)[c]	76.3 (2.5)[d]

*Entries are mean values with standard deviations in parentheses and the mean values with the same superscript letter were not significantly different ($p > 0.05$).

It is known that GICs increase their strengths with time due to constant salt-bridge formation [34]. To confirm if the F-modified GIC still follows the pattern that most GICs exhibit, we examined both CS and antibacterial activity of the cements after aging in water for 1 h, 1, 3, 7, 14 and 30 d. The result in Fig. 6 showed that the experimental cement showed 30% increase in CS after 30-day aging in water, compared to 1 h aging. The result also showed that the cement had 14% and 25% increase after 1 and 7 d aging, compared to 1 h aging. The result is consistent with those reported elsewhere [34, 35]. Meanwhile no statistically significant changes in the S. mutans viability were found during the 30 d aging. The result implies that F might not leach out of the cement; otherwise both CS and antibacterial activity would show a decreasing trend.

Table III shows the property comparison among the cements with and without F and commercial Fuji II LC. As shown in Table III, the F-containing cement was 26% in YS, 15% in modulus, 14% in CS, 19% in DTS and 23% in FS lower than the cement without F. On the other hand, the F-containing cement was much higher (19% higher) in antibacterial activity than the cement without F. As compared to commercial Fuji II LC, the cement with F showed 8% in CS, 5% in modulus, 4% in FS, 0% in CS higher but 3% in DTS and 18% in S. mutans viability lower than Fuji II LC. The results suggest that the F-containing antibacterial cement may be used as an alternative for Fuji II LC due to enhanced antibacterial activity and comparable mechanical strengths.

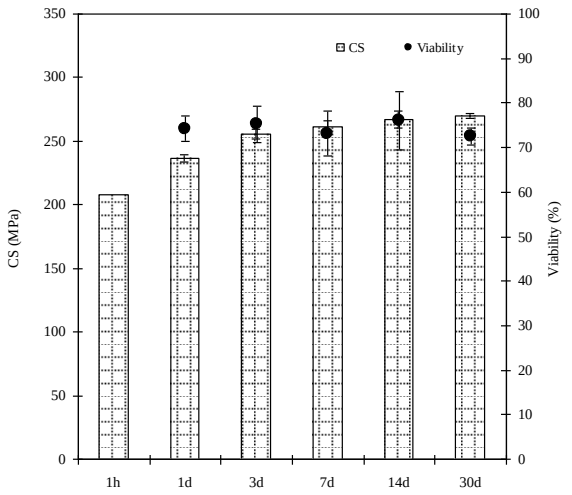

Fig. 6. Effect of aging on CS and S. mutans viability of the experimental cement.

TABLE III. PROPERTY COMPARISON AMONG EXPERIMENTAL CEMENTS WITH F, WITHOUT F AND FUJI II LC

Property	EXPGIC	EXPGICF	Fuji II LC
YS [MPa]	205.7 (3.6)	152.8 (4.5)	141.2 (1.9)
M [GPa]	8.52 (0.15)	7.23 (0.23)	6.89 (0.38)
CS [MPa]	275.3 (5.9)	236.4 (8.2)[a]	236.2 (3.4)[a]
DTS [MPa]	51.2 (3.5)	41.6 (4.1)[b]	42.8 (0.9)[b]
FS [MPa]	71.4 (4.6)	55.2 (5.1)[c]	53.3 (2.1)[c]
Viability (%)	92.1 (4.1)[d]	74.2 (2.9)	90.9 (0.9)[d]

*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 developed a novel antibacterial glass-ionomer cement. The new cement showed a

significant antibacterial activity, accompanying with an initial CS reduction. The effects of F content, GM grafting ratio, P/W ratio, P/L ratio, human saliva, and aging in water on CS and S. mutans viability were significant. The human saliva did not affect the antibacterial activity of the cement. The result also indicates that the cement may have a long-lasting antibacterial function.

ACKNOWLEDGMENT

This work was partially sponsored by NIH grant DE020614 and Dr. Gregory at Indiana University School of Dentistry was acknowledged for providing laboratory for the antibacterial tests.

REFERENCES

[1] M.P. Muller, C. MacDougall, M. Lim, “Antimicrobial surfaces to prevent healthcare-associated infections: a systematic review”, J. Hospital Infection, vol., 92 (1), pp. 7-13, 2016.

[2] T.E. Donovan, R. Marzola, W. Becker, D.R. Cagna, F. Eichmiller, J.R. McKee, J.E. Metz, J.P. Albouy, “Annual review of selected scientific literature: Report of the committee on scientific investigation of the American Academy of Restorative Dentistry”, J. Prost. Dent., vol. 114 (6), pp. 756-809, 2015.

[3] K.J. Anusavice, Phillips’ Science of dental Materials, 11th ed., St Louis, MO: Sauders, 2003.

[4] I.A. Mjor, J.E. Dahl, J.E. Moorhead, “Placement and replacement of restorations in primary teeth”, Acta. Odontol. Scand., vol. 60, pp. 25-28, 2002.

[5] H. Forss, E. Widstrom, “Reasons for restorative therapy and longevity of restorations in adults”, Acta. Odontol. Scand., vol. 62, pp. 82-86, 2004.

[6] J. Manhart, F. Garcia-Godoy, R. Hickel, “Direct posterior restorations: clinical results and new developments”, Dent. Clin. North. Am., vol. 46, pp. 303-39, 2002.

[7] V. Deligeorgi, I.A. Mjor, N.H. Wilson, “An overview of reasons for the placement and replacement of restorations”, Prim. Dent. Care., vol. 8, pp. 5-11, 2001.

[8] R.G. Craig, J.M. Power, Restorative Dental Materials, 11th ed., St Louis, MO: Mosby-Year Book, Inc., 2002, pp. 614-618.

[9] A. Wiegand, W. Buchalla, T. Attin, “Review on fluoride-releasing restorative materials - Fluoride release and uptake characteristics, antibacterial activity and influence on caries formation”, Dent. Mater., vol. 23, pp. 343–362, 2007.

[10] P.W. Osinaga, R.H. Grande, R.Y. Ballester, M.R. Simionato, C.R. Delgado Rodrigues, A. Muench, “Zinc sulfate addition to glass-ionomer-based cements: influence on physical and antibacterial properties, zinc and fluoride release”, Dent. Mater., vol. 19, pp. 212-217, 2003.

[11] Y. Takahashi, S. Imazato, A.V. Kaneshiro, S. Ebisu, J.E. Frencken, F.R. Tay, “Antibacterial effects and physical properties of glass-ionomer cements containing chlorhexidine for the ART approach”, Dent. Mater., vol. 22, pp. 467-452, 2006.

[12] K. Yamamoto, S. Ohashi, M. Aono, T. Kokubo, I. Yamada, J. Yamauchi, “Antibacterial activity of silver ions implanted in SiO2 filler on oral streptococci”, Dent. Mater., vol. 12, pp. 227-229, 1996.

[13] T. Syafiuddin, H. Hisamitsu, T. Toko, T. Igarashi, N. Goto, A. Fujishima, T. Miyazaki, “In vitro inhibition of caries around a resin composite restoration containing antibacterial filler”, Biomaterials, vol. 18, pp. 1051-1057, 1997.

[14] S. Imazato, R.R. Russell, J.F. McCabe, “Antibacterial activity of MDPB polymer incorporated in dental resin”, J. Dent., vol. 23, pp. 177-181, 1995.

[15] G. Lu, D. Wu, R. Fu, “Studies on the synthesis and antibacterial activities of polymeric quaternary ammonium salts from dimethylaminoethyl methacrylate”, React. & Funct. Polym., vol. 67, pp. 355–366, 2007.

[16] S.B. Lee, R.R. Koepsel, S.W. Morley, K. Matyjaszewski, Y. Sun, A.J. Russell, “Permanent, nonleaching antibacterial surfaces. 1. Synthesis by atom transfer radical polymerization”, Biomacromolecules, vol. 5, pp. 877-882, 2004.

[17] F. Li, Z.G. Chai, M.N. Sun, F. Wang, S. Ma, L. Zhang, M. Fang, J.H. Chen, “Anti-biofilm effect of dental adhesive with cationic monomer”, J. Dent. Res., vol. 88, pp. 372-376, 2009.

[18] N. Beyth, I. Yudovin-Farber, R. Bahir, A.J. Domb, E.I. Weiss, “Antibacterial activity of dental composites containing quaternary ammonium polyethylenimine nanoparticles against Streptococcus mutans”, Biomaterials, vol. 27, pp. 3995-4002, 2006.

[19] D. Xie, Y. Weng, X. Guo, J. Zhao, R.L. Gregory, C. Zheng, “Preparation and evaluation of a novel glass-ionomer cement with antibacterial functions”, Dent. Mater., vol. 27, pp. 487-496, 2011.

[20] S. Imazato, N. Ebi, Y. Takahashi, T. Kaneko, S. Ebisu, R.R.B. Russell, “Antibacterial activity of bactericide-immobilized filler for resin-based restoratives”, Biomaterials, vol. 24, pp. 3605-3609, 2003.

[21] N. Ebi, S. Imazato, Y. Noiri, S. Ebisu, “Inhibitory effects of resin composite containing bactericide-immobilized filler on plaque accumulation”, Dent. Mater., vol. 17, pp. 485-491, 2001.

[22] J.B. Jones, J.M. Young, “Carcinogenicity of lactones III: The reactions of unsaturated 4-lactones with l-cysteine”, J. Med. Chem., vol. 11, pp. 1176, 1968.

[23] E. Lattmann, S. Dunn, S. Niamsanit, N. Sattayasai, “Synthesis and antibacterial activities of 5-hydroxy-4-amino-2(5H)-furanones”, Bioorg. Med. Chem. Lett., vol. 15, pp. 919-921, 2005.

[24] E. Lattmann, J. Coombs, H.M.R. Hoffmann, “Paranofuranones via lewis acid mediated hetero-Diels-Alder reactions of 4-Furan-2(5H)-ones. A convergent route to the manoalide substructure”, Synthesis, pp. 171-177, 1996.

[25] D. Martinelli, G. Grossmann, U. Sequin, H. Brandl, R. Bachofen, “Effects of natural and chemically synthesized furanones on quorum sensing in Chromobacterium violaceum”, B.M.C. Microbiol., vol. 4, pp. 25, 2004.

[26] W.S. Sung, H.J. Jung, I.S. Lee, D.G. Lee, “Antimicrobial effect of furonoel against human pathogenic bacteria and fungi”, J. Microbiol. Biotechnol., vol.16(3), pp. 349-354, 2006.

[27] R.T.A. Mayadunne, G. Moad, E. Rizzardo, “Multiarm organic compounds for use as reversible chain-transfer agents in living radical polymerizationd”, Tetra. Lett., vol. 43, pp. 6811-6814, 2002.

[28] D. Xie, J.G. Park, M. Faddah, J. Zhao, H.K. Khanijoun, “Novel amino acid-constructed polyalkenoates for dental glass-ionomer restoratives”, J. Biomater. Appl., vol. 21, pp. 147-165, 2006.

[29] L. Howard, Y. Weng Y, D. Xie, “Preparation and evaluation of a novel star-shapedpolyacid-constructed dental glass–ionomer system”, Dent. Mater., vol. 30, pp. 644-653, 2014.

[30] A.D. Wilson, “Resin-modified glass-ionomer cement”, Int. J. Prosthodont., vol. 3(5), pp. 425-429, 1990.

[31] D. Xie, Y. Yang, J. Zhao, J.G. Park, J.T. Zhang, “A novel comonomer-free light-cured glass-ionomer system for reduced cytotoxicity and enhanced mechanical strength”, Dent. Mater., vol. 23, pp. 994-1003, 2007.

[32] D. Xie, J.G. Park, J. Zhao, “Synthesis and preparation of novel 4-arm star-shape poly(carboxylic acid)s for improved light-cured glass-ionomer cements”, Dent. Mater., vol. 23, pp. 395-403, 2007.

[33] S. Crisp, B.G. Lewis, A.D. Wilson, (1976). Characterization of Glass-ionomer Cements. 2. Effect of the Powder: Liquid Ratio on the Physical Properties, J. Dent., vol. 4(6), pp. 287–290, 1976.

[34] C.L. Davidson, I.A. Mjor, Advances in glass—ionomer cements. Chicago, IL: Quintessence Publishing Co; 1999.

[35] M.A. Cattani-Lorente, V. Dupuis, F. Moya, J. Payan, J.M. Meyer, “Comparative study of the physical properties of a polyacid-modified composite resin and a resin-modified glass ionomer cement”, Dent. Mater., vol. 15, pp. 21-32, 1999.