Preparation and evaluation of a novel antibacterial glass-ionomer cement

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ABSTRACT
A novel antibacterial glass-ionomer cement has been developed. Compressive strength (CS) and S. mutans viability were used to evaluate the mechanical strength and antibacterial activity of the formed cement. Compressive yield strength (YS), modulus (M), diametral tensile strength (DTS) and flexural strength (FS) were also determined. All the formulated antibacterial cements showed a significant antibacterial activity, accompanying with an initial CS reduction. The effect of the synthesized antibacterial polymer loading was significant. Increasing loading from 1% to 20% significantly decreased the S. mutans viability from 3% to 50% and also reduced the initial CS (325 MPa) of the formed cements from 19% to 75%. The cement with 5% antibacterial polymer loading showed 142 MPa, 6.9 GPa, 224 MPa, 52 MPa, and 62 MPa in YS, M, CS, DTS and FS, respectively, as compared to 170, 7.1, 325, 60 and 87 for the experimental cement without antibacterial polymer addition and 141, 6.9, 236, 42 and 53 for Fuji II LC. It was also found that the chlorine-containing antibacterial cement showed better CS values than the bromine-containing cement, with no significant difference in antibacterial activity. The antibacterial cement also showed a similar antibacterial activity to Streptococcus mutans, lactobacillus, Staphylococcus aureus and Staphylococcus epidermidis. The human saliva did not affect the antibacterial activity of the cement. The thirty-day aging study indicates that the cements may have a long-lasting antibacterial function.

Keywords: Dihalomalealdehydic Acid Derivative; Antibacterial Polymer; S. mutans Viability; Glass-Ionomer Cement; CS

1. INTRODUCTION
Secondary caries is found to be the main reason for the restoration failure of dental restoratives including resin composites and glass-ionomer cements [1-4]. 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 [4]. 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 [5-9]. 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 [5-9]. 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 [10-14]. 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 [12], use of methacryloxyethyl cetyl ammonium chloride as a com-
ponent for antibacterial bonding agents [16,17], addition of quaternary ammonium polyethylenimine nanoparticles into composite resins [18-23], and incorporation of polyQAS (PQAS) into glass-ionomer cements (GICs) [24]. All these studies found that the QAS-containing materials did exhibit significant antibacterial activities. 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 [25,26]. Recently furanone derivatives have been found to have strong antitumor [27,28] and antibacterial functions [29]. 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 [30]. The similar compounds were also found to show an inhibitory effect on bacterial quorum-sensing [31], probably due to the structural similarity to autoinducers in bacteria. The exact antibacterial mechanism is still unclear and under investigation. Here we hypothesize that incorporating a newly synthesized furanone derivative to GICs would make a GIC with an antibacterial function, allowing us to explore them in dental applications.

The objective of this study was to synthesize and characterize a new furanone derivative and its containing polyacid, use the polyacid to formulate the 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.

2. MATERIALS AND METHODS

2.1. Materials
Acrylic acid (AA), glycolic acid (GA), 2,3-dichloromalealdehydic acid (DCA), 2,3-dibromomalealdehydic acid (DBA), dipentaerythritol, 2-bromoisoobutyryl bromide (BIBB), 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%), sulfuric acid, pyridine, diethyl ether, dioxane, N,N-dimethylformamide (DMF), toluene, hexane and tetrahydrofuran (THF) were used as received from Sigma-Aldrich Co. (Milwaukee, WI) without further purifications. Light-cured glass-ionomer cement Fuji II LC and Fuji II LC glass powders (Batch # 0704101) were used as received from GC Corp (Japan).

2.2. Synthesis and Characterization

2.2.1. Synthesis of the DCA Methacrylate Derivative
To a solution containing DCA (0.5 mol), toluene and sulfuric acid (1% by mole), glycolic acid (0.55 mol) in toluene was added. The reaction was run at 90 - 100°C for 3 - 4 h until no more water came out. Then toluene was removed using a rotary evaporator. The formed DCAGA was purified by washing with sodium bicarbonate and distilled water. After freeze-drying, DCAGA (0.5 mol) was mixed with GM (0.52 mol) and pyridine (3%, by weight) in DMF to form DCAGAGM. The mixture was reacted at 50°C for 8 h [32], followed by washing with hexane and diethyl ether and drying in a vacuum oven prior to use. DBAGAGM was synthesized similarly. The synthesis scheme is shown in Figure 1(a).

2.2.2. Synthesis of the Poly(Acrylic Acid-Co-DCAGAGM)
The linear poly(acrylic acid-co-DCAGAGM) copolymer or abbreviated as CAP (chlorine-containing antibacterial polymer) was prepared following our published procedures [33]. Briefly, to a flask containing a solution of AA (0.1 mol) and DCAGAGM (0.1 mol) in THF, AIBN (1% by mole) in THF was added. After the reaction was run under N2 purging at 60°C for 18 h, the polymer was precipitated with diethyl ether, followed by drying in a vacuum oven. The synthesis scheme is shown in Figure 1(b).

2.2.3. Synthesis of the GM-Tethered Star-Shape Poly(Acrylic Acid)
The GM-tethered 6-arm star-shaped poly(acrylic acid) (PAA) was synthesized similarly as described in our previous publication [34]. Briefly, dipentaerythritol (0.06 mol) in 200 ml THF was used to react with BIBB (0.48 mol) in the presence of TEA (0.35 mol) to form the 6-arm initiator. t-BA (0.078 mol) in 10 ml dioxane was then polymerized with the 6-arm initiator (1% by mole) at 120°C in the presence of CuBr (3%)-PMDETA (3%) catalyst complex via atom-transfer radical polymerization. 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) [34]. The GM-tethered star-shaped PAA was recovered by precipitation from diethyl ether, followed by drying in a vacuum oven at room temperature. The synthesis scheme is shown in Figure 1(c).

2.2.4. Characterization
The chemical structures of the synthesized furanone derivatives were characterized by Fourier transform-infrared (FT-IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. The proton NMR (1HNMR) spectra were obtained on a 500 MHz Bruker NMR spectrometer (Bruker Avance II, Bruker BioSpin Corporation,
(a) Synthesis of functional antibacterial monomer

\[
\begin{align*}
\text{OH} & \quad \text{CO}_2\text{H} \\
\text{X} & \quad \text{X} \\
\text{H}_3\text{CO} & \quad \text{O}
\end{align*}
\]

(1) \rightarrow

\[
\begin{align*}
\text{OH} & \quad \text{CO}_2\text{H} \\
\text{X} & \quad \text{X} \\
\text{H}_3\text{CO} & \quad \text{O}
\end{align*}
\]

(2) \rightarrow

\[
\begin{align*}
\text{OH} & \quad \text{CO}_2\text{H} \\
\text{X} & \quad \text{X} \\
\text{H}_3\text{CO} & \quad \text{O}
\end{align*}
\]

Where \( X = \text{Cl or Br} \)

(b) Synthesis of functional antibacterial polymer

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\text{X} & \quad \text{X} \\
\text{H}_3\text{CO} & \quad \text{OH}
\end{align*}
\]

(3) \rightarrow

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\text{X} & \quad \text{X} \\
\text{H}_3\text{CO} & \quad \text{OH}
\end{align*}
\]

Where \( m:n = 1 \)

(c) Synthesis of in situ photo-curable star-shaped poly(acrylic acid)

\[
\begin{align*}
\text{O} & \quad \text{Br} \\
\text{X} & \quad \text{X} \\
\text{H}_3\text{CO} & \quad \text{Br}
\end{align*}
\]

(4) \rightarrow

\[
\begin{align*}
\text{O} & \quad \text{Br} \\
\text{X} & \quad \text{X} \\
\text{H}_3\text{CO} & \quad \text{Br}
\end{align*}
\]

(5) \rightarrow

\[
\begin{align*}
\text{O} & \quad \text{Br} \\
\text{X} & \quad \text{X} \\
\text{H}_3\text{CO} & \quad \text{Br}
\end{align*}
\]

(6) \rightarrow

\[
\begin{align*}
\text{O} & \quad \text{Br} \\
\text{X} & \quad \text{X} \\
\text{H}_3\text{CO} & \quad \text{Br}
\end{align*}
\]

Where \( x:y = 1 \)

Figure 1. Schematic diagrams for synthesis of antibacterial monomer, antibacterial polymer and in situ polymerizable 6-arm star-shaped polymer: (a) DCAGAGM synthesis; (b) poly(AA-co-DCAGAGM) synthesis; (c) Synthesis of the 6-arm star-shaped poly(AA) with pendant methacrylates.
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).

2.3. Evaluation

2.3.1. Cement Sample Preparation for Strength and Antibacterial Tests

The experimental cements were formulated with a two-component system (liquid and powder) [34]. The liquid was formulated with the light-curable star-shaped PAA, water, 0.9% CQ (photo-initiator, by weight) and 1.8% DMAEMA (activator). The polymer/water (P/W) ratio = 70:30 (by weight). Fuji II LC glass powder was either used alone or mixed with the synthesized poly(AA-co-DCA-gAGM) or CAP to formulate the cements, where the polymer loading ratio = 1, 3, 5, 7, 10, or 20% (by weight) of the glass and the powder/liquid (P/L) ratio = 2.7 (by weight).

Specimens were fabricated at room temperature according to the published protocol [34]. 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, 30 W, 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°C for 24 h prior to testing, unless specified.

2.3.2. 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 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/πr², where P = the load at fracture and r = the radius of the cylinder. DTS was determined from the relationship DTS = 2P/πdr, 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 = 3P/2bd², 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.

2.3.3. MIC Test for the Synthesized Antibacterial Monomers

The minimal inhibitory concentration (MIC) of the synthesized antibacterial monomers was determined following the published protocol with a slight modification [24]. 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 monomer 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 monomer ranged from 1.563 to 2 × 10⁴ µg/ml. The microtiter plate was then inoculated with S. mutans suspension (cell concentration = 5 × 10⁵ CFU/ml) and incubated at 37°C for 48 h prior to MIC testing. The absorbance was measured at 595 nm via a microplate reader (SpectraMax 190, Molecular Devices, CA) to assess the cell growth. Chlorhexidine (CHX) and dimethylsulfoxide were used as positive and negative controls, respectively [24]. The concentration of CHX was used in the same range as shown for the monomers. The final concentration of dimethylsulfoxide was 2% (v/v). Triple replica was used to obtain a mean value for each material.

2.3.4. Antibacterial Test

The antibacterial test was conducted following the published procedures [24]. S. mutans was mainly used to evaluate the antibacterial activity of the studied cements throughout the study. Other bacteria including lactobacillus, Staphylococcus aureus (S. aureus) and Staphylococcus epidermidis (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 10⁵ 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°C for 48 h under anaerobic condition with 5% CO₂, 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

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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.

2.3.5. Saliva Effect Test
Human saliva, obtained from a healthy volunteer, was centrifuged for 15 min at 12,000 g to remove debris [25]. After the supernatant was filtered with a 0.45 μm sterile filter, the filtrate was stored in a −20°C freezer prior to use. The sterilized cement specimen (see Section 2.3.4) was incubated in a small tube containing 1 ml of saliva at 37°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 in Section 2.3.4.

2.3.6. Aging of the Specimens
The specimens for both CS and antibacterial activity aging tests were conditioned in distilled water at 37°C for 1 day, 3 days, 7 days and 30 days, followed by direct testing for CS (see Section 2.3.2 for details) and incubating with S. mutans for 48 h for antibacterial testing (see Section 2.3.4 for details).

2.3.7. 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 or formulations in each group. A level of α = 0.05 was used for statistical significance.

3. RESULTS

3.1. Characterization

Figure 2 shows the FT-IR spectra of GA, DCA, DCAGA, GM and DCAGAGM. The disappearance of the peak (cm⁻¹) at 3362 for pseudo hydroxyl group on pseudo ester and appearance of the two new peaks at 1810 and 1768 for the carbonyl groups from pseudo ester and GA ester as well as a broad COOH peak at 3650-2500 confirmed the formation of DCAGA. The disappearance of the broad carboxylic acid peak at 3650-2500 and appearance of 3480 (OH), 3001-2930 (CH₃), 1786, 1742 and 1720 (C=O from pseudo ester, GA ester and methacrylate), and 1638 and 1620 (C=C on methacrylate and pseudo ester) confirmed the formation of DCAGAGM. Table 1 shows the ¹H NMR chemical shifts (ppm) for GA, DCA, DCAGA, GM and DCAGAGM. All the new chemical shifts shown in Table 1 for DCAGAGM confirmed the formation of DCAGAGM.

3.2. Evaluation

Figure 3 shows the effect of the chlorine-containing antibacterial polymer or CAP content on CS (a) and S. mutans viability (b) of the cements. Both mean CS and S. mutans viability values decreased with increasing CAP content, where there were no statistically significant differences between 1% and 3% for CS and among Fuji II LC, 0% and 1%, between 1% and 3%, and between 7% and 10% for the S. mutans viability (p > 0.05). The CAP addition significantly decreased both CS and S. mutans viability, with a reduction of 19% to 75% for CS and 3% to 50% for the S. mutans viability.

Table 2 shows the MIC of DCA, DBA, DCAGAGM, DBAGAGM and CHX to S. mutans, lactobacillus, S. aureus and S. epidermidis. The MIC values ranged from 9.36 to 74.9 μg/ml for DCA and DBA, 4.68 to 150 for DCAGAGM and DBAGAGM, and 1.563 to 6.25 for CHX.

Table 3 shows the effect of both CAP and bromide-containing antibacterial polymer (BAP) on CS and S. mutans viability of EXPGIC. Like those CAP in Figure 3, increasing the loading of BAP decreased the CS values of the cements and S. mutans viability. By comparison, it is clear that at the same loading the BAP cements showed statistically lower CS values than the CAP cements, although both were not statistically significant different from each other in antibacterial activity.

Figure 4 shows the effect of the CAP cement aging on CS and S. mutans viability. After 30-day aging in water, the cement with 7% CAP addition showed a significant CS increase (26%). However, no statistically significant changes were found in the S. mutans viability for the cement.

Figure 5 shows the effect of the CAP content on the viability of four bacteria including S. mutans, lactobacillus, S. aureus and S. epidermidis. Increasing CAP decreased the viability of all the bacteria, where there were no statistically significant differences in viability between 7% and 10% for S. mutans, between 5% and 7% for lactobacillus and between 10% and 20% for S. epidermidis (p > 0.05). With 20% of CAP addition, the viability of all four bacteria decreased to 47%.

Figure 6 shows the effect of human saliva 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.

Table 4 shows the property comparison among the cements with 0%, 5% and 7% of CAP addition and Fuji II LC. As compared to the cement with 0% CAP, the cements with 5% and 7% CAP showed a decrease in all the measured strengths. The decreases of 16% to 27%, 3% to 11%, 31% to 41%, 13% to 20% and 29% to 32% were observed, respectively, in yield strength (YS), compressive modulus (M), CS, diametral tensile strength (DTS) and flexural strength (FS), among which CS and
Figure 2. FT-IR spectra for GA, DCA, DCAGA, GM and DCAGAGM: (a) GA; (b) DCA; (c) DCAGA; (d) GM and (e) DCAGAGM.

Figure 3. Effect of the CAP content on CS and S. mutans viability of the experimental cements: (a) Effect on CS and (b) Effect on the S. mutans viability. MW of the 6-arm poly(acrylic acid) = 17,530 Daltons; Filler = Fuji II LC or Fuji II LC + CAP; Grafting ratio = 50%; P/L ratio = 2.7; P/W ratio = 70:30. Fuji II LC was used as control. Specimens were conditioned in distilled water at 37°C for 24 h, followed by direct testing for CS or/and incubating with S. mutans for 48 h for antibacterial testing.

Table 1. The characteristic chemical shifts from the 1HNMR spectra.

<table>
<thead>
<tr>
<th>Material</th>
<th>The characteristic chemical shifts (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>11.55 (-COOH), 4.57 (-CH₂-) and 3.90 (-OH)</td>
</tr>
<tr>
<td>DCA</td>
<td>6.25 (-CH) and 3.45 (-OH)</td>
</tr>
<tr>
<td>DCAGA</td>
<td>11.55 (-COOH), 6.25 (-CH) and 4.90 (-CH₂-)</td>
</tr>
<tr>
<td>GM</td>
<td>6.10 and 5.70 (H₂C=C-), 5.45 (-CH), 3.2-3.3 (-CH₂-), 2.65-2.8 (-CH₂-) and 1.90 (-C=CH-)</td>
</tr>
<tr>
<td>DCAGAGM</td>
<td>6.20 (-CH), 6.10 and 5.70 (H₂C=C-), 4.90 (-CH₂- on GM), 4.15 (-CH₂- on GM), 3.70 (-CH- on GM), 3.50 (-CH₂- on GA), 3.25 (-OH) and 1.90 (-C=CH-)</td>
</tr>
</tbody>
</table>
Table 2. MIC values of the materials used in the study.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>S. mutans</th>
<th>lactobacillus</th>
<th>S. aureus</th>
<th>S. epidermidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCA</td>
<td>9.36</td>
<td>18.7</td>
<td>18.7</td>
<td>18.7</td>
</tr>
<tr>
<td>DBA</td>
<td>37.4</td>
<td>74.9</td>
<td>37.4</td>
<td>37.4</td>
</tr>
<tr>
<td>DCAGAGM</td>
<td>18.7</td>
<td>18.7</td>
<td>9.36</td>
<td>4.68</td>
</tr>
<tr>
<td>DBAGAGM</td>
<td>74.9</td>
<td>150</td>
<td>74.9</td>
<td>74.9</td>
</tr>
<tr>
<td>Chlohexidine</td>
<td>1.56</td>
<td>6.25</td>
<td>3.12</td>
<td>3.12</td>
</tr>
</tbody>
</table>

1DCA, DBA, DCAGAGM and DBAGAGM are the abbreviations of antibacterial compounds, which can be found under Materials and Methods; 2MIC values (μg/ml) were measured as described in the text.

Table 3. Effect of CAP and BAP on CS and S. mutans viability of EXPGIC3.

<table>
<thead>
<tr>
<th>Polymer [%]</th>
<th>CS</th>
<th>S. mutans viability [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAP</td>
<td>BAP</td>
</tr>
<tr>
<td>1</td>
<td>263.1 (4.2)3</td>
<td>231.2 (2.3)</td>
</tr>
<tr>
<td>3</td>
<td>252.8 (5.1)3</td>
<td>201.4 (4.2)</td>
</tr>
<tr>
<td>5</td>
<td>224.7 (7.8)</td>
<td>179.1 (0.8)</td>
</tr>
<tr>
<td>7</td>
<td>192.5 (13)</td>
<td>145.2 (2.1)</td>
</tr>
<tr>
<td>10</td>
<td>163.4 (4.3)</td>
<td>121.3 (3.6)</td>
</tr>
</tbody>
</table>

1The formulations were the same as those described in Figure 3, except that CAP contains chlorine but BAP contains bromine; 2Polymer = antibacterial polymer (% by weight); 3Entries are mean values with standard deviations in parentheses and the mean values with the same superscript letter were not significantly different (p > 0.05). Specimens were conditioned in distilled water at 37°C for 24 h, followed by direct testing for CS or/and incubating with S. mutans for 48 h for antibacterial testing.

Figure 4. Effect of aging on CS and S. mutans viability of the experimental cements:
The formulations were the same as those described in Figure 3, except for the CAP content = 7%. Specimens were conditioned in distilled water at 37°C for 1, 7, 14 and 30 days, followed by direct testing for CS or/and incubating with S. mutans for 48 h for antibacterial testing.
Figure 5. Effect of the CAP content on the viability of different bacteria after culturing with the experimental cements: The formulations were the same as those described in Figure 3. Specimens were conditioned in distilled water at 37°C for 24 h, followed by direct testing for CS or incubating with *S. mutans* for 48 h for antibacterial testing.

Figure 6. Effect of human saliva on the *S. mutans* viability after culturing with the cements: The formulations were the same as those described in Figure 3. Specimens were incubated in human saliva at 37°C for 2 h, followed by incubating with *S. mutans* for 48 h for antibacterial testing.

FS showed more reduction. A significant decrease with a 19% - 28% reduction was observed in the *S. mutans* viability. Commercial GIC Fuji II LC showed the similar YS, M and CS values to but lower DTS and FS values.
than the experimental cement with 5% CAP. Fuji II LC also showed the similar S. mutans viability to the cement with 0% CAP.

4. DISCUSSION

Figure 3 shows the effect of the CAP content on CS and S. mutans viability of the cements. It is apparent that with CAP addition the cement showed a decrease in CS and S. mutans viability. The cements lost 19% to 75% of its original CS value (325 MPa) with 1% to 20% CAP addition, among which the cements with 5% and 7% CAP showed a 30% to 40% loss but the values were still close to 200 MPa. The loss of CS can be attributed to the incorporated CAP because hydrophobic CAP did not contribute any strength enhancement to the cements. Regarding the S. mutans viability, CAP significantly increased the antibacterial activity of the cement. With 1% to 20% CAP addition, the S. mutans viability was reduced from 3% to 50%, among which the cements with 5% and 7% CAP showed a 19% to 28% reduction. Considering the feasibility of GICs applied in dental clinics, the CS value below 200 MPa may not be well acceptable because the CS values of most commercially available light-cured GICs are in the range of 180 to 240 MPa [5,35]. Therefore, the cements with 5% and 7% CAP addition were chosen to evaluate other properties.

Due to the smaller size of chlorine, we hypothesized that CAP might favor the mechanical strength as compared to BAP, although we did not know if their antibacterial activity would be different. The result in Table 3 shows that the CAP cements were statistically significantly higher in CS than the BAP cements, indicating that our hypothesis was correct, i.e., smaller chlorine favors CS. However, no significant differences in antibacterial activity were found between CAP and BAP cement (see Table 3), despite the fact that DCA and DCAGAGM were higher in inhibition of all the four bacteria strains than DBA and DBAGAGM based on MIC test (see Table 2). The result suggests that the CAP cement might be a better choice for cement formulation than the cement on behalf on CS and antibacterial tests.

It is well known that GICs increase their strengths with time due to constant salt-bridge formations [36]. To confirm if the CAP-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 day, 7 days, 14 days and 30 days. The result in Figure 4 shows that the cements with 7% CAP showed 26% increase in CS after 30-day aging in water and no changes in the S. mutans viability were found. The reason can be attributed to the fact that CAP is a copolymer of acrylic acid and DCAGAGM. It is known that the carboxylic acid group plays a key role in GIC setting and salt-bridge formation. CAP not only provided antibacterial function but also supplied carboxyl groups for salt-bridge formation. The carboxyl groups helped the polymer to firmly attach to the glass fillers. The results also imply that the CAP did not leach out of the cement; otherwise both CS and antibacterial activity would show a decreasing trend.

As stated in Introduction, 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 other implant infections. To examine the antibacterial activity of CAP on these bacteria, we compared the viability of all the four bacteria after incubating with the cements. The result in Figure 5 shows that no statistically noticeable differences in viability were found among the four bacteria, even though the absolute values were different from one another. Increasing the CAP content significantly decreased the viability of all the bacteria, indicating that CAP can also kill other bacteria.

Figure 6 shows the effect of human saliva on the S. mutans viability after culturing with the CAP 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 [25,26]. The reduction was attributed to the interaction between positive charges

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Table 4. Comparison of properties of the experimental cements with and without CAP1.

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<tr>
<td>0</td>
<td>170.1 (5.6)</td>
<td>7.12 (0.12)</td>
<td>325.3 (4.2)</td>
<td>60.1 (0.9)</td>
<td>87.2 (2.2)</td>
<td>93.2 (1.3)</td>
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<tr>
<td>5</td>
<td>142.3 (6.9)</td>
<td>6.86 (0.17)</td>
<td>224.7 (7.9)</td>
<td>52.2 (2.3)</td>
<td>62.1 (3.3)</td>
<td>75.7 (2.2)</td>
</tr>
<tr>
<td>7</td>
<td>124.6 (5.9)</td>
<td>6.31 (0.05)</td>
<td>192.5 (13)</td>
<td>48.3 (1.1)</td>
<td>58.9 (4.6)</td>
<td>66.9 (4.0)</td>
</tr>
<tr>
<td>Fuji II LC</td>
<td>141.2 (1.9)</td>
<td>6.89 (0.38)</td>
<td>236.2 (3.4)</td>
<td>42.8 (0.9)</td>
<td>53.3 (2.1)</td>
<td>90.9 (0.9)</td>
</tr>
</tbody>
</table>

1The formulations were the same as those described in Figure 3; 2Polymer = CAP; 3YS = CS at yield; 4M = compressive modulus; 5DTS = diametral tensile strength; 6FS = flexural strength; 7Fuji II LC = commercial GIC; Entries are mean values with standard deviations in parentheses and the mean values with the same superscript letter were not significantly different (p > 0.05). Specimens were conditioned in distilled water at 37°C for 24 h, followed by direct testing for all the strengths and incubating with S. mutans for 48 h for antibacterial testing.

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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 [25,26]. Unlike QAS or PQASA, CAP does not carry any charges. That may be why the CAP-modified cements did not show any reduction in antibacterial activity after treating with saliva.

Finally we compared YS, M, CS, DTS, FS and the S. mutans viability of the cements having 0%, 5% and 7% CAP and Fuji II LC. As shown in Table 2, the CAP-modified cements were 16% - 17% in YS, 3.6% - 11% in modulus, 31% - 41% in CS, 13% - 20% in DTS and 29% - 32% in FS lower than the cement without CAP addition. On the other hand, the CAP-modified cements were much higher (19% and 28% higher) in antibacterial activity than the cement without CAP addition. As compared to commercial GIC Fuji II LC, the cement with 5% CAP showed the similar YS, M and CS, higher DTS (22% higher) and FS (17% higher), and lower S. mutans viability (20% lower) values.

5. CONCLUSION
We have developed a novel antibacterial glass-ionomer cement. All the modified cements showed a significant antibacterial activity, accompanying with an initial CS reduction. The effect of the synthesized antibacterial polymer loading was significant. Increasing loading significantly enhanced antibacterial activity but reduced the initial CS of the formed cements. The CAP cement showed better CS values than the BAP cement, with no significant difference in antibacterial activity. The antibacterial cements showed a similar antibacterial activity to S. mutans, lactobacillus, S. aureus and S. epidermidis. The human saliva did not affect the antibacterial activity of the cement. The 30-day aging study indicates that the antibacterial cement may have a long-lasting antibacterial function. Future work will include optimizing the formulations and evaluating all major mechanical properties such as hardness, wear-resistance, fracture toughness and bonding to enamel and dentin, physical properties such as working and setting times, conversion, shrinkage, component leaching, water-sorption and solubility, biological properties such as cytotoxicity to pulp and gingival cells, clinically-relevant properties such as colorization, thermal expansion and fluoride release, and antibacterial test against Gram-negative bacteria.

6. ACKNOWLEDGEMENTS
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REFERENCES

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LIST OF THE ABBREVIATIONS

CS: compressive strength;
M: modulus;
DTS: diametral tensile strength;
FS: flexural strength;
GIC: glass-ionomer cement;
PAA: poly(acrylic acid);

gA: glycolic acid;
DCA: 2,3-dichloromalealdehydic acid;
GM: glycidyl methacrylate;
DBA: dibromomalealdehydic acid;
CAP: chlorine-containing antibacterial polymer;
BAP: bromine-containing antibacterial polymer.