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Incorporating 2,3-Dichloromalealdehydic Acid-coated Inorganic Fillers to Enhance Antibacterial Function of A Dental Cement

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***Abstract*—An antibacterial 2,3-dichloromalealdehydic acid derivative was synthesized and covalently attached onto the zirconium oxide filler surfaces. The formed antibacterial fillers were then mixed into a light-curable dental glass ionomer cement formulation. Compressive strength and bacterial viability were used to evaluate the modified cements. Results showed that almost all the modified cements exhibited higher antibacterial activity along with decreased compressive strength, as compared to the unmodified control. With increasing antibacterial 2,3-dichloromalealdehydic acid derivative content and modified filler loading, yield strength, modulus and compressive strength were decreased. On the contrary, the strengths were increased with increasing powder/liquid ratio. On the other hand, with increasing 2,3-dichloromalealdehydic acid derivative content, filler loading and a powder/liquid ratio, antibacterial activity was enhanced.**

Keywords—antibacterial; light-cured glass ionomer cement; compressive strength; bacterial viability

I. INTRODUCTION

Recently Dental glass-ionomer cements (GICs), one of the two main filling restoratives – composites and GICs, show many attractive properties over composites [1-2]. These properties include but are not limited to direct bond to tooth and base metals, thermal compatibility with enamel and dentin, low shrinkage, fluoride release, etc [1-2]. Although GICs shows anticariogenic and somehow antibacterial properties due to fluoride release, their antibacterial property is still not potent enough to prevent secondary caries formation due to the fact that fluoride release is dose-dependent and would run out with time [3, 4]. It is known that secondary caries is one of the main reasons to the restoration failure of dental restoratives [5, 6]. Therefore, making antibacterial dental restoratives is attractive to dental community. In the last two decades, materials scientists have made numerous efforts at improving antibacterial functions of dental restoratives. There have been two main strategies to prevent secondary caries caused by oral bacteria. The first one is to incorporate low molecular weight antibacterial agents into dental restorative formulations, where the mechanism is based upon release or slow release of these low molecular weight agents. Such agents include but are not limited to various antibiotics, chlorhexidine, zinc ion, silver ion and iodine [7-10]. However, release or slow-release of antibacterial agents can suffer from a mechanical property reduction of the restoratives over time, have short-term effectiveness but long-term run-out of the releasing agents, produce possible toxicity of agents to surrounding tissues, and enhance a chance for antibiotic-resistant bacteria formation [7-10]. The second one is to incorporate high molecular weight antibacterial polymers or covalently link antibacterial agent to restoratives or devices [11-15], where the mechanism is based on kill by contact [14]. The latter is considered to be more effective than the former. One typical example is to incorporate organic quaternary ammonium salts into the restoratives [11-16]. The quaternary ammonium salt-containing materials are found to show a broad spectrum of antimicrobials and also be able to kill or inhibit bacteria that are resistant to other types of cationic antibacterial compounds [17]. There are numerous studies of using the quaternary ammonium salt derivatives for dental restoratives include applying polymerizable methacryloyloxydodecyl pyridinium bromide in cement resins [14], using curable methacryloxylethyl cetyl ammonium chloride in antibacterial bonding agents [18, 19], adding polyethylenimine quaternary ammonium nanoparticles to composites [15], and incorporating polymerizable quaternary ammonium bromide derivatives with different chain lengths into glass-ionomer cements [16]. The results showed that all the above quaternary ammonium salt-modified dental restoratives have exhibited significant antibacterial activities. Another example is to incorporate furanone-derivatized agents into restoratives. The furanone derivatives have shown strong antitumor [20, 21] and antibacterial functions [22]. Recently these derivatives were successfully incorporated into dental glass-ionomer

cements [23] and dental composites [24], resulting in the promising outcomes [23, 24]. However, after the derivatives were directly mixed with the curable resin and cured *in situ*, potential unreacted antibacterial derivatives could be leached out to the surrounding tissues due to incomplete monomer to polymer conversion [25]. Therefore, in this study, we proposed to use inorganic zirconium oxide particles as a delivery vehicle to deliver antibacterial agent by covalently coating a cured antibacterial polymer on zirconium oxide particle surface, followed by thoroughly washing the fillers to remove the uncoated derivatives and then incorporating into the system, to reduce the leachable. Zirconium oxide is known to be radio-opaque and biocompatible [26]. Using it as a delivery vehicle could also enhance radio-opacity of cements.

The purpose of this study was to covalently coat an antibacterial furanone derivative onto radio-opaque zirconium oxide particles, use these coated particles to formulate an antibacterial cement for enhanced antibacterial activity, and evaluate the compressive strength and antibacterial function of the formed cement.

II. Material and Methods

A. Materials

Triethylene glycol dimethacrylate, acrylic acid, p-toluenesulfonic acid monohydrate, 2,3-dichloromalealdehydic acid, 2-hydroxyethyl acrylate, potassium persulfate, toluene, methanol, sodium bicarbonate, and zirconium oxide (amorphous) were received from Sigma-Aldrich Co. (Milwaukee, WI) and used without further purifications. Commercial glass-ionomer cement Fuji II LC and its corresponding glass powders were used as received from GC America Inc (Alsip, IL).

B. Synthesis and Characyterization

Zirconium oxide particle surface coating was carried out with the following four steps: (1) Synthesis of 2,3-dichloromalealdehydic acid-hydroxyethyl acrylate (DH). To a mixture of 2,3-dichloromalealdehydic acid, toluene and p-toluenesulfonic acid monohydrate, 2-hydroxyethyl acrylate in toluene was added [27]. After the mixture was run at 90-100 ^{o}C for 4 h, toluene was removed using a rotary evaporator. The formed DH was purified by washing with sodium bicarbonate and distilled water, followed by freeze-drying. (2) Surface activation with acrylic acid. zirconium oxide particles were dispersed in acrylic acid with ultrasonic vibration for 10 min [28], followed by heating at 70 ^{o}C overnight. (3) Antibacterial resin fixation on particle surface. This process was conducted by immersing acrylic aicd-activated zirconium oxide particles in a mixture of DH and triethylene glycol dimethacrylate in methanol, followed by removing methanol with a rotary evaporator. (4) Covalently crosslinking antibacterial resin on the particle surfaces. This was completed by dispersing the particles in distilled water containing potassium persulfate, followed by heating at 70 ^{o}C for 3 h, washing, filtering and freeze-drying.

The coated and uncoated zirconium oxide surfaces were characterized with Fourier transform-infrared (FT-IR). FT-IR spectra were acquired on a FT-IR spectrometer (Mattson Research Series FT/IR 1000, Madison, WI).

C. Evaluation

A two-component system was used to formulate the antibacterial experimental cement. The glass fillers were prepared by blending the antibacterial-coated zirconium oxide particles with Fuji II LC glass powders using a vortex mixer. Fuji II LC liquid was used directly with the formulated glass fillers to form the cement per manufacturer's instruction. A powder/liquid ratio of 2.7 (wt/wt) was applied throughout the study unless specified.

Samples were prepared by mixing the liquid with the glass fillers thoroughly at room temperature, according to the published protocol [29]. The cylindrical samples were made in a glass tubing with dimensions of 8 mm in length x 4 mm in diameter for compressive strength (CS) and 4 mm in diameter x 2 mm in thickness for bacterial viability tests, respectively. All the prepared samples were illuminated with a blue light device (EXAKT 520 Blue Light Polymerization Unit, EXAKT Technologies, Inc., Oklahoma City, OK) for 2 min, removed from the mold, and conditioned in distilled water at 37 °C for 24 h prior to testing.

CS was tested on a screw-driven mechanical tester (QTest QT/10, MTS Systems Corp., Eden Prairie, MN), with a crosshead speed of 1 mm/min [29]. CS was calculated with a formula $P/\pi r^2$, where P = the load at fracture and r = the radius of the cylinder. Yield strength (YS) and modulus (M) were obtained from the stress-strain curves of the CS test.

The bacterial viability was measured based on the protocol elsewhere [30]. 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. Four bacteria species including *Streptococcus mutans (S.M.), Staphylococcus aureus (S.A.)*, *Pseudomonas aeruginosa* (*P.A.)* and *Escherichia coli (E.C.)* 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 in each test.

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

Fig. 1 shows a set of FT-IR spectra for zirconium oxide (a), acrylic acid-coated zirconium oxide (b) and resin-coated zirconium oxide (e). Spectrum a shows a peak at 3418 cm^{-1} hydroxyl groups from adsorbed water on zirconium oxide particles [31]. It has been reported that this type of water on any manufactured ceramic particles are hardly removed [32]. Spectrum b shows strong peaks at 1728 for carbonyl group and at 1654 for carbon-carbon double bonds, which confirmed successful coating of acrylic acid on zirconium oxide particle surface by forming intra chelating bonds between carboxylic acid and zirconium oxide. Spectrum c shows the peaks at 1788 for intra ester group on 2,3-dichloromalealdehydic acid and at 1729 for ester group on 2-hydroxyethyl acrylate, which confirmed that DH was successfully coated on the acrylic acid-coated zirconium oxide particle surfaces.

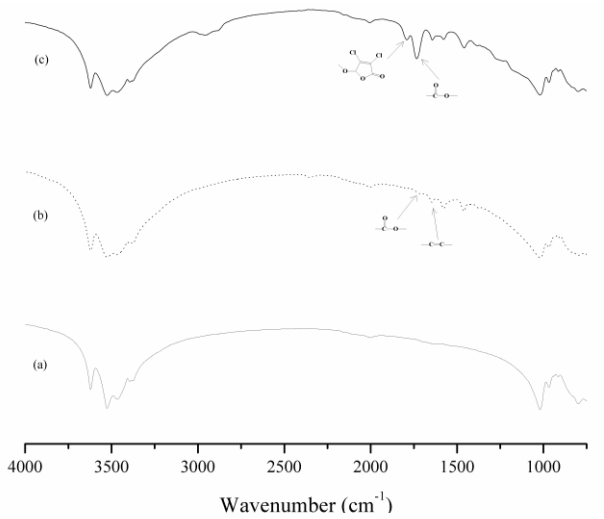

Fig. 1. FT-IR spectra of coated and non-coated particles

B. Evaluation

Fig. 2 shows the effects of antibacterial moiety content on CS and *S.M.* viability. The mean CS (MPa) was in the decreasing order of control > 15% = 10% = 5%, where no significant differences were found among 5%, 10% and 15% ($p > 0.05$). The mean *S.M.* viability was in the decreasing order of Control > 5% > 10% > 15%. With increasing antibacterial moiety content, no changes were found among the modified cements with commercial control showing a significantly higher CS than the modified cement. On the other hand, addition of DH moiety does exhibit a significantly strong antibacterial function.

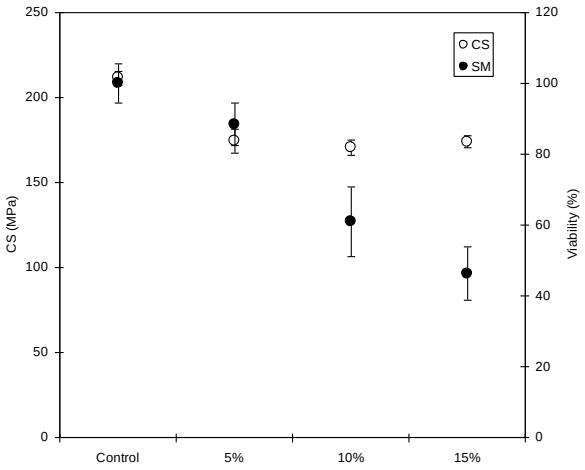

Fig. 2. Effect of DH moiety content on CS and S.M. viability

Fig. 3 shows the effects of antibacterial zirconium oxide filler loading on CS and *S.M.* viability. The mean CS was in the decreasing order of control > 10% > 20% > 30% > 40% > 50%. The mean *S.M.* viability was in the decreasing order of control > 10% > 20% > 30% > 40% > 50%, where no significant difference was found between 40% and 50%. With increasing antibacterial filler loading, CS was significantly decreased. This is probably because the added zirconium oxide fillers are amorphous, which did not provide any strength enhancement functions to the system. On the other hand, the bacterial viability was significantly decreased with increasing filler loading. This can be easily explained as the reason that increasing antibacterial filler loading increased the coated antibacterial moiety contents, thus enhancing the antibacterial activity of the modified cement.

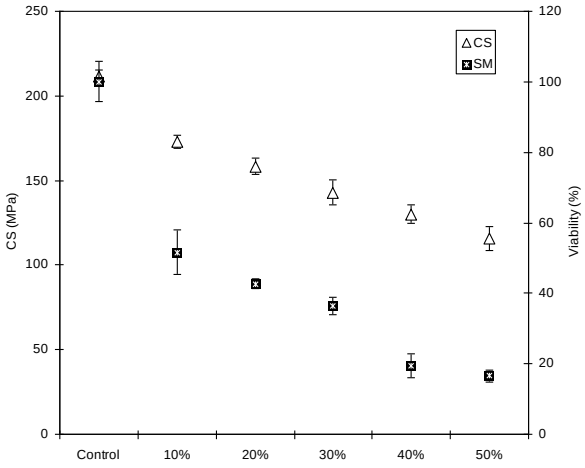

Fig. 3. Effect of antibacterial resin-coated filler content on CS and S.M. viability

Fig. 4 shows the effects of P/L ratio on CS and *S.M.* viability. The mean CS was in the decreasing order of control > 3.3 > 3.0 > 2.7 > 2.4 by weight. The mean *S.M.* viability was in the decreasing order of control > 2.4 > 2.7 > 3.0 > 3.3. Clearly with increasing

P/L ratio CS was significantly increased with the lowest at 2.4 but the highest at 3.3 but still was lower than control. On the other hand, bacterial viability was significantly reduced or in other words, antibacterial activity was significantly increased. In glass-ionomer cement formulations, glass fillers are inorganic components which often enhance compressive strengths but organic resins often enhance plastic properties or reduce compressive strength [1, 33]. Since the added zirconium oxide fillers are amorphous and coated with antibacterial organic resins, theoretically speaking, they would not enhance compressive strength but with increasing quantity, the compressive strength showed an increasing trend. Meanwhile with increasing total filler loading (high P/L ratio), the corresponding antibacterial moiety content was also increased, thus resulting in enhanced antibacterial activity.

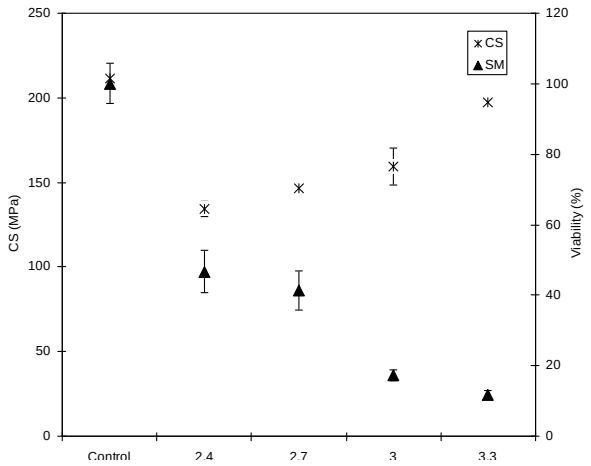

Fig. 4. Effect of P/L ratio on CS and S.M. viability

Table 1 shows the effect of antibacterial fillers on yield strength (YS), modulus (M) and compressive strength (CS). For antibacterial moiety content, increasing DH moiety content decreased YS, M and CS, where there were significant differences in both YS and CS among control and other modified cements. However, control did show statistically significant higher M than any other modified cements, where no significant differences were among the other modified cements. For antibacterial filler loading, increasing filler loading significantly decreased YS, M and CS, with control showing significantly higher values than any modified cements and the cement with 50% loading showing the lowest values. The reason was the same as discussed previously. For P/L ratio, increasing P/L ratio or total filler content increased YS, M and CS. The explanation was similar to that discussed previously. Inorganic fillers often promote stiffness including yield strength and modulus of the materials [1, 34]. Zirconium oxide is an inorganic filler which shows brittleness. When mixing inorganic fillers with organic resins, plastic deformation decreases but stiffness increases. Yield strength and modulus are a symbol for stiffness. That is why by adding zirconium oxide fillers both YS and M were significantly increased.

TABLE I. EFFECTS OF ZIRCONIUM OXIDE LOADING AND P/L RATIOS ON CS

	YS (MPa)	M (GPa)	CS (MPa)
Effect of DH moiety content			
Control	145.9 (5.2)	6.39 (0.15)	211.4 (4.1)
5%	137.2 (2.0)	6.27 (0.09)	174.6 (6.9)
10%	132.9 (7.2)	6.18 (0.18)	170.5 (4.5)
15%	129.8 (3.1)	6.29 (0.13)	174.0 (3.8)
Effect of antibacterial resin-coated filler content			
10%	133.4 (6.2)	6.43 (0.32)	172.7 (3.7)
20%	122.3 (7.2)	6.19 (0.13)	158.4 (4.8)
30%	111.8 (6.4)	5.59 (0.21)	142.8 (7.5)
40%	102.9 (3.4)	5.29 (0.15)	130.1 (5.6)
50%	91.5 (2.2)	4.96 (0.20)	115.6 (7.1)
Effect of P/L ratio			
2.4	101.5 (4.3)	5.29 (0.11)	134.2 (4.5)
2.7	108.4 (2.0)	5.83 (0.09)	146.2 (2.8)
3.0	123.8 (11)	6.39 (0.16)	159.6 (10)
3.3	149.4 (8.2)	6.75 (0.37)	197.2 (3.6)

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

TABLE II. EFFECT OF P/L RATIO ON VIABILITY OF FOUR BACTERIA (%)

	Control	2.4	2.7	3.0	3.3
S. M.	100 (5.7)	46.7 (6.1)	41.3 (5.6)	17.3 (1.4)	11.7 (1.2)
E. C.	100 (11)	63.1 (1.9)	37.2 (2.0)	35.5 (3.0)	21.1 (0.4)
S. A.	100 (6.8)	74.5 (7.7)	65.9 (5.2)	44.2 (3.4)	20.6 (0.2)
P. A.	100 (2.7)	73.1 (7.8)	58.8 (2.9)	44.0 (6.6)	30.2 (0.9)

*Specimens were cultured with bacteria for 48 h before testing

Table 2 shows the effect of P/L ratio on the viability of four bacterial species. From the results, it is clear that increasing P/L ratio decreased bacterial viability. Different bacterial species showed different responses to antibacterial cement resins. From 2.7 to 3.6, *S.M., E.C.*, *S.A.* and *P.A.* showed 58%, 80%, 71% and 64% decrease in bacterial viability, respectively. As compared with control, *S.M., E.C.*, *S.A.* and *P.A.* showed 73%, 85%, 77% and 69% decrease in bacterial viability, respectively. The result indicates that the responses from different bacteria species to the antibacterial compound are different. *E.C.* and *S.A.* are more vulnerable to the antibacterial compound DH than *S.M.* and *P.A.*

IV. CONCLUSIONS

An antibacterial 2,3-dichloromalealdehydic acid-containing resin was covalently coated onto the zirconium oxide filler surfaces. The coated fillers were

incorporated into dental cement. Compressive strength and bacterial viability were used to evaluate the modified cements. Results showed that almost all the modified cements exhibited higher antibacterial activity along with decreased compressive strength, as compared to the unmodified control. With increasing antibacterial 2,3-dichloromalealdehydic acid derivative content and modified filler loading, yield strength, modulus and compressive strength were decreased. On the contrary, the strengths were increased with increasing powder/liquid ratio. On the other hand, with increasing antibacterial 2,3-dichloromalealdehydic acid derivative content, filler loading and powder/liquid ratio, antibacterial activity was enhanced.

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