DEVELOPING NOVEL ANTIBACTERIAL DENTAL FILLING COMPOSITE RESTORATIVES

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ABSTRACT

Caneli, Gulsah M.S.B.M.E., Purdue University, May 2020. Developing Novel Antibacterial Dental Filling Composite Restoratives. Major Professor: Dong Xie.

A novel antimicrobial dental composite system has been developed and evaluated. Both alumina and zirconia filler particles were covalently coated with an antibacterial resin and blended into a composite formulation, respectively. Surface hardness and bacterial viability were used to evaluate the coated alumina filler-modified composite. Compressive strength and bacterial viability were used to evaluate the coated zirconia filler-modified composite. Commercial composite Kerr was used as control. The specimens were conditioned in distilled water at 37 °C for 24 h prior to testing. Four bacterial species Streptococcus mutans, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli were used to assess the bacterial viability. Effects of antibacterial moiety content, modified particle size and loading, and total filler content were investigated.

Chapter 2 describes how we studied and evaluated the composite modified with antibacterial resin-coated alumina fillers. The results showed that almost all the modified composites exhibited higher antibacterial activity along with improved surface hardness, as compared to unmodified one. Increasing antibacterial moiety content, particle size and loading, and total filler content generally increased surface hardness. Increasing antibacterial moiety, filler loading, and total filler content increased antibacterial activity. On the other hand, increasing particle size showed a negative impact on antibacterial activity. The leaching tests indicate that the modified experimental composite showed no leachable antibacterial component to bacteria.

Chapter 3 describes how we studied and evaluated the composite modified with antibacterial resin-coated zirconia fillers. The results showed that almost all the modified composites exhibited higher antibacterial activity along with decreased compressive strength, as compared to the unmodified control. It was found that with increasing antibacterial moiety content and modified filler loading, yield strength, modulus and compressive strength of the composite were decreased. In addition, the strengths of the composite were increased with increasing powder/liquid ratio. On the other hand, with increasing antibacterial moiety content, filler loading and powder/liquid ratio, antibacterial activity was enhanced.

In summary, we have developed a novel antibacterial dental composite system for improved dental restoratives. Both composites modified with the antibacterial resin-coated alumina and zirconia fillers have demonstrated significant antibacterial activities. The composite modified with the alumina fillers showed improved hardness values, but the composite modified with the zirconia fillers showed decreased compressive strength values. It appears that the developed system is a non-leaching antibacterial dental composite.

CHAPTER 1. INTRODUCTION

1.1 Background

Long-lasting dental restoratives are welcomed to both dental clinics and scientific community, because they can reduce patients' dental office visit and resultant physical pains as well as expenses [1]. Clinically attractive dental filling restoratives should not only have mechanical and physical properties that are comparable to natural teeth but also exhibit antibacterial property that can prevent teeth from forming secondary caries [2, 3]. Secondary caries is a type of caries that is generated by oral bacteria after dental restorations. It is a tooth demineralization produced by plaque bacteria such as Streptococcus mutans in the presence of fermentable carbohydrates. The process occurs at the interface between the cavity preparation and the restoration [3]. Secondary caries has been reported to be the main reason to the dental restoration failure [1, 4]. Therefore, prevention of secondary caries is very important in restorative dentistry. So far, two main strategies have been used to reduce or prevent secondary caries caused by bacteria. One is to incorporate low molecular weight antibacterial compounds into dental restorative formulations. The antibacterial mechanism is based upon release or slow-release of these low molecular weight compounds. Such compounds include but are not limited to various antibiotics, chlorhexidine, zinc ion, silver ion and iodine [5, 6]. However, release or slow-release of compounds can suffer from a mechanical property reduction of the restoratives over time, short-term effectiveness but long-term run-out of the releasing compound, possible toxicity of the compound to surrounding tissues, and an enhanced chance for antibiotic-resistant bacteria formation due to decreasing concentration of the released compound [5, 6]. The other strategy is to incorporate high molecular weight antibacterial polymers or covalently link

antibacterial compound to restoratives or devices [7,8]. The antibacterial mechanism of this strategy is based on "kill by contact" [9]. This strategy seems a more effective strategy, as compared to release or slow release. One of the typical examples is to incorporate organic quaternary ammonium salts into the restoratives [7, 10]. The quaternary ammonium salt-containing materials have been found to show a broad spectrum antimicrobial activity and kill or inhibit bacteria that are resistant to other types of cationic antibacterial compounds [11]. The examples of using the quaternary ammonium salt derivatives for dental restoratives include applying polymerizable methacryloyloxydodecyl pyridinium bromide in 12 composites [9], using curable methacryloxylethyl cetyl ammonium chloride in antibacterial bonding agents [12,13], adding polyethylenimine quaternary ammonium nanoparticles to composites [14], and incorporating polymerizable quaternary ammonium bromide derivatives with different chain lengths into glass-ionomer cements [10]. The results showed that all the above quaternary ammonium salt-modified dental restoratives did exhibit significant antibacterial activities. Another example of using this strategy is to incorporate furanone-derivatized compounds into restoratives. The furanone derivatives have shown strong antitumor [15, 16] and antibacterial functions [17]. Recently these derivatives were incorporated into dental glass-ionomer cements [18] and dental composites [19], resulting in the promising outcomes [18,19]. The formulated cements and composites showed a significant bacterial inhibition that is comparable to those formulated with the quaternary ammonium salt derivatives [10]. Unfortunately, our unpublished lab results have shown that by in situ polymerization the cured composites showed to be leachable due to incomplete monomer-to-polymer conversion [6, 20]. Therefore, in this study, we proposed to use alumina particles and/or zirconia particles as a delivery vehicle to deliver antibacterial agent by covalently coating a cured antibacterial polymer on alumina particle surface, to achieve a goal of formulating an antibacterial composite with an enhanced antibacterial function without leaching antibacterial moieties.

1.2 Hypothesis and Objectives

It is our hypothesis that incorporating crystalline alumina or non-crystalline zirconia filler particles in which surfaces were covalently coated with an antibacterial compound - DA derivative, into current dental composite restorative, would provide a novel route for formulating a novel antibacterial dental restorative. The objectives of the study in this thesis were to:

1. Synthesize and characterize the antibacterial resin-coated alumina and zirconia fillers.

2. Formulate the composites with the coated antibacterial fillers.

3. Evaluate the hardness and/or compressive strength of the formed composites.

4. Evaluate the antibacterial activity of the formed composites.

CHAPTER 2. COATING OF ALUMINA PARTICLE SURFACE FOR IMPROVED ANTIBACTERIAL DENTAL PROPERTY

2.1 Introduction

Long-lasting dental restoratives are attractive to both dental clinics and scientific community, because they can reduce patients' dental office visit and resultant physical pains as well as expenses [1]. Clinically attractive dental filling restoratives should not only have mechanical and physical properties that are comparable to natural teeth but also exhibit antibacterial property that can prevent teeth from forming secondary caries [2, 4]. Secondary caries is a type of caries that is generated by oral bacteria after dental restorations. It is a tooth demineralization produced by plaque bacteria such as Streptococcus mutans in the presence of fermentable carbohydrates. The process°C occurs at the interface between the cavity preparation and the restoration [21]. Secondary caries has been reported to be the main reason to the dental restoration failure [1, 4]. Therefore, prevention of secondary caries is very important in restorative dentistry. So far, two main strategies have been used to reduce or prevent secondary caries caused by bacteria. One is to incorporate low molecular weight antibacterial compounds into dental restorative formulations. The antibacterial mechanism is based upon release or slow-release of these low molecular weight compounds. Such compounds include but are not limited to various antibiotics, chlorhexidine, zinc ion, silver ion and iodine [5, 6]. However, release or slow-release of compounds can suffer from a mechanical property reduction of restoratives over time, short-term effectiveness but long-term run-out of the releasing compound, possible toxicity of the compound to surrounding tissues, and an enhanced chance for antibiotic-resistant bacteria formation due to decreasing concentration of the released compound [5, 6]. The other strategy

is to incorporate high molecular weight antibacterial polymers or covalently link antibacterial compound to restoratives or devices [7,8]. The antibacterial mechanism of this strategy is based on "kill by contact" [9]. This strategy seems a more effective strategy, as compared to release or slow release. One of the typical examples is to incorporate organic quaternary ammonium salts into restoratives [7, 10]. The quaternary ammonium salt-containing materials have been 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 [11]. The examples of using the quaternary ammonium salt derivatives for dental restoratives include applying polymerizable methacryloyloxydodecyl pyridinium bromide in composites [9], using curable methacryloxylethyl cetyl ammonium chloride in antibacterial bonding agents [12,13], adding polyethylenimine quaternary ammonium nanoparticles to composites [14], and incorporating polymerizable quaternary ammonium bromide derivatives with different chain lengths into glass-ionomer cements [10].. The results showed that all the above quaternary ammonium salt-modified dental restoratives did exhibit significant antibacterial activities. Another example of using this strategy is to incorporate furanone-derivatized compounds into restoratives. The furanone derivatives have shown strong antitumor [15, 16] and antibacterial functions [17]. Recently these derivatives were incorporated into dental glass-ionomer cements [18] and dental composites [19], resulting in the promising outcomes [18]. The formulated cements and composites showed a significant bacterial inhibition that is comparable to those formulated with the quaternary ammonium salt derivatives [10]. Unfortunately, our unpublished lab results have shown that by in situ polymerization the cured composites showed leachable due to incomplete monomer-to-polymer conversion [6, 20]. Therefore, in this study, we proposed to use alumina particles as a delivery vehicle to deliver antibacterial agent by covalently coating a cured antibacterial polymer on alumina particle surface, to formulate an antibacterial composite with an enhanced antibacterial function and improved mechanical hardness without leaching antibacterial moieties.

The purpose of this study was to covalently coat an antibacterial furanone derivative onto crystalline alumina particles, use these coated particles as a delivery vehicle to formulate a novel antibacterial dental composite for improved antibacterial activity and surface hardness, and evaluate the surface hardness and antibacterial property of the formed composite.

2.2 Materials and Methods

2.2.1 Materials

Bisphenol A glycidyl methacrylate, triethylene glycol dimethacrylate, acrylic acid, 2-hydroxyethyl acrylate, p-toluenesulfonic acid monohydrate, 3,4-dichloromalealdehydic acid, toluene, sodium bicarbonate, Y-(trimethoxysilyl)propyl methacrylate, potassium persulfate, camphoroquinone, and 2-(dimethylamino)ethyl methacrylate, and alumina particles with different sizes were received from Sigma-Aldrich Co. (Milwaukee, WI) and used without further purifications. The Herculite-XRV (particle = 0.7 microns, untreated) glass fillers were received as a gift from Kavo Kerr Dental Specialties (Orange, CA).

2.2.2 Synthesis and Characterization

2.2.2.1 Synthesis of 3,4-dichloromalealdehyic acid hydroxyethyl acrylate

To a solution containing 3,4-dichloromalealdehydic acid (DA, 0.1 mol), toluene and p-toluenesulfonic acid monohydrate (1% by mole), 2-hydroxyethyl acrylate (HA, 0.11 mol) in toluene was added [22]. After the mixture was run at 90-100 °C for 4 h, toluene was removed using a rotary evaporator. The formed DAHA was purified by washing with sodium bicarbonate and distilled water, followed by freeze-drying. The synthesis scheme is shown in Figure 2.1.

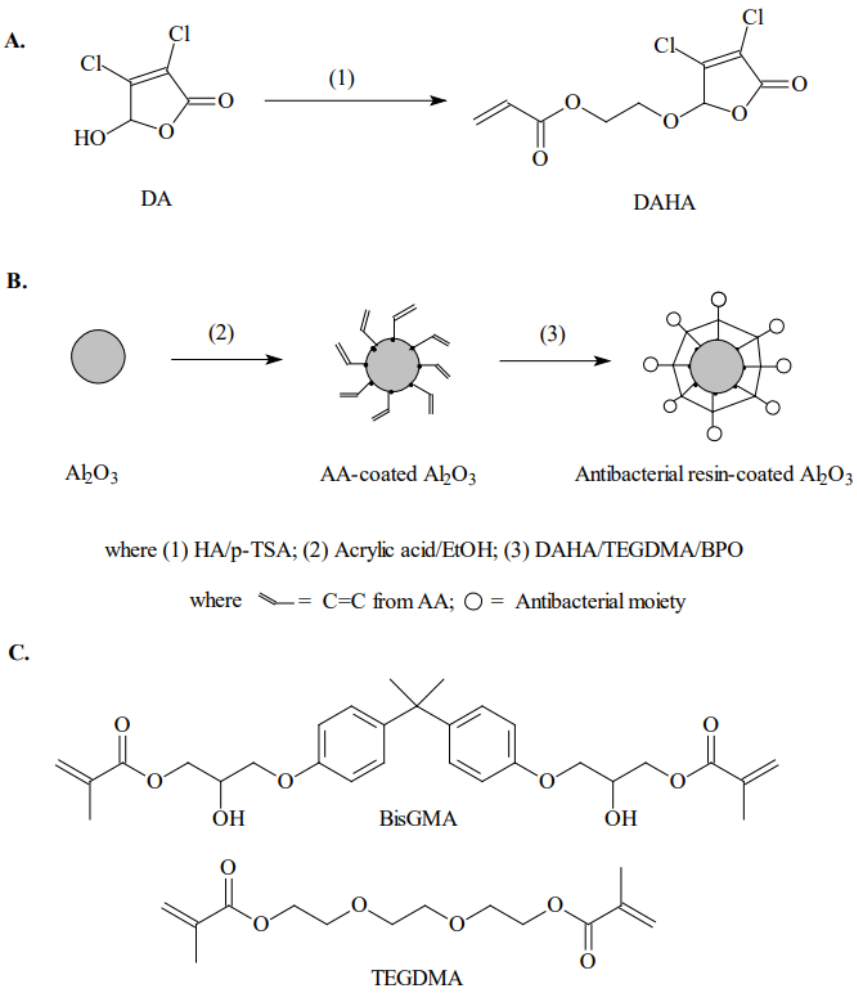


Fig. 2.1. Schematic diagrams for synthesis of DAHA and as well as oligomer structures: A. DAHA synthesis; B. Antibacterial resin-coated alumina particle preparation; C. BisGMA and TEGDMA structures

2.2.2.2 Surface coating of antibacterial resin onto alumina particles

Surface coating was accomplished with the following three steps: (1) Surface activation with acrylic acid. Briefly alumina particles were dispersed in acrylic acid with ultrasonic vibration for 10 min [23], followed by heating at 70 °C overnight, washing with methanol and filtering. (2) Fixation of antibacterial agent on particle surface. This process was conducted by immersing acrylic acid-activated alumina

particles in a mixture of DAHA and triethylene glycol dimethacrylate in methanol, followed by removing methanol with a rotary evaporator(3). Covalently coating antibacterial agent on the particle surfaces. This process was completed by dispersing the particles in distilled water containing potassium persulfate, followed by heating at 70 °C for 3 h, washing, filtering and freeze-drying. The coating scheme is shown in Figure 2.1.

2.2.2.3 Characterization

The alumina particle surfaces were characterized with Fourier transform-infrared (FT-IR) and thermal gravity analysis (TGA). FT-IR spectra were acquired on a FT-IR spectrometer (Mattson Research Series FT/IR 1000, Madison, WI). The thermal decomposition history of selected alumina particles was determined on a thermogravimetric analyzer (Mettler Toledo, Columbus, OH) at a heating rate of 10°C/min under nitrogen.

2.2.3 Evaluation

2.2.3.1 Specimen preparation for evaluations

The experimental composites were formulated with a two-component (powder and liquid) system [20]. The glass powders (silicon dioxide, Herculite XRV) were treated with y-(trimethoxysilyl)propyl methacrylate, following the published protocol [20]. The treated glass powders were then blended with the antibacterial resin-coated alumina particles using a vortex mixer. The liquid portion was formulated with bisphenol, glycidyl methacrylate (BisGMA, 50% wt/wt), triethylene glycol dimethacrylate (TEGDMA, 50%), camphoroquinone (photoinitiator, 1%), and 2-(dimethylamino)ethyl methacrylate (activator, 2%), following the protocol elsewhere [20]. The composite without any antibacterial resin-coated alumina filler addition is

named as “Kerr”. A glass filler content at 75% (wt/wt) was applied throughout the study unless specified.

Specimens were prepared by mixing the liquid with the glass fillers thoroughly at room temperature, according to the published protocol [20]. Briefly, the cylindrical specimens were made in a glass ring with dimensions of 4 mm in diameter x 2 mm in thickness, having a transparent microscope glass slide on each side, for surface hardness, bacterial viability and cell viability tests. All the prepared specimens 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.

2.2.3.2 Hardness test

The hardness test was performed on a micro-hardness tester (LM-100, LECO Corporation, MI) using a diamond indenter with 25 g load and 30 s dwell time. Knoop hardness number (KHN) was averaged from six readings for each sample.

2.2.3.3 Bacterial viability test

The bacterial viability test was carried out based on the protocol described elsewhere [8]. 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. Four bacterial species including Streptococcus mutans (S. mutans), Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa) and Escherichia coli (E. coli) 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 µL of the above bacterial suspension, 3 µL of a fluorescent green/red (1:1 v/v) stain (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 (green) and dead (red) bacteria were imaged with an inverted fluorescence microscope (EVOS FL, AMG, Mill Creek, WA, USA). A bacterial suspension without disks was used as control and viable bacteria counts from 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.

The specimen elute test was conducted based on the following protocol. Briefly, a disc specimen was sterilized with 70% ethanol and sterile phosphate buffer saline (PBS), followed by immersing in a 96-well plate containing tryptic soy broth at 37 °C for 48 h under 5% CO_2. After the specimen was removed, the bacterial suspension in tryptic soy broth was added into each well and incubated at 37 °C for 48 h under 5% CO_2. Then the viability was analyzed by counting from the recorded images with using ImageJ software.

2.2.3.4 3T3 Mouse fibroblast viability test

The 3T3 mouse fibroblast viability test was conducted based on the protocol described elsewhere [24]. Briefly, three steps were followed as below: (1) Culturing cells: 3T3 cells were cultured at 37 °C for 48 h in an air atmosphere containing 5% CO_2 and 95% relative humidity, with Dulbecco's modified Eagle's medium (DMEM, Hyclone Laboratories, Inc. Logan, UT) containing low glucose, supplemented with 10% heat-inactivated fetal bovine serum (Hyclone Laboratories), 4 mM L-glutamine (Hyclone Laboratories), 100 U/ml penicillin (Sigma-Aldrich, St. Louis, MO), 50 µg/ml gentamicin (Invitrogen Life Technologies, Carlsbad, CA) and 2.5 µg/ml amphotericin B fungizone (Lonza,Walkersville, MD). (2) Elute preparation of the test materials: The disc specimen was sterilized with 70% ethanol and sterile phosphate buffer saline (PBS), followed by immersing in a 48-well plate containing 300 µl serum minus DMEM for 48 h. (3) The water soluble tetrazolium salt-1 (WST-1) test: The

cells were plated in a 96-well plate at 2 x 10^4 cells per well in 100 µl of DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin. After incubation at 37 °C overnight, the medium was replaced with 100 µl of the fresh medium containing different concentrations of eluate (50%). The cells were then incubated for 48 h before the WST-1 testing. The positive control was serum minus DMEM with untreated cells and the negative control was serum minus DMEM without cells. The WST-1 test was carried out by adding 10 µl of WST-1 reagent (Roche Diagnostics, Indianapolis, IN) and 90 µl of serum minus DMEM into a well and then incubating the plate at 37 °C for 2 h. The absorbance of the solution was measured at 450 nm using a microplate reader (Perkin Elmer 1420 Multilabel Counter, Victor 3, Akron, OH). Cell viability (%) was obtained by the equation: cell viability (%) = (absorbance of the sample eluate - absorbance of the negative control) / (absorbance of the positive control - absorbance of the negative control) × 100. Triplicate samples were used to obtain a mean value for each material.

2.2.3.5 Statistical Analysis

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

2.3 Results and Discussion

2.3.1 Characterization

Figure 2.2 shows the TGA weight-loss curves for Al_2O_3, acrylic acid (AA)-coated Al_2O_3 and antibacterial resin-coated Al_2O_3 particles. The quantitative transition points for weight-loss are: (a) Al_2O_3: 2.5% weight loss due to absorbed moisture or water. (b) AA-coated Al_2O_3: 2.5% weight loss from 30 to 150°C (absorbed water)

and 11% loss from 150 to 500°C (AA coating). (c) Antibacterial resin-coated Al_2O_3: 2.5% weight loss from 30 to 200°C (absorbed water), 11% loss from 200 to 350°C (AA coating), and 12% loss from 350 to 500 °C (cross-linked antibacterial resin coating).

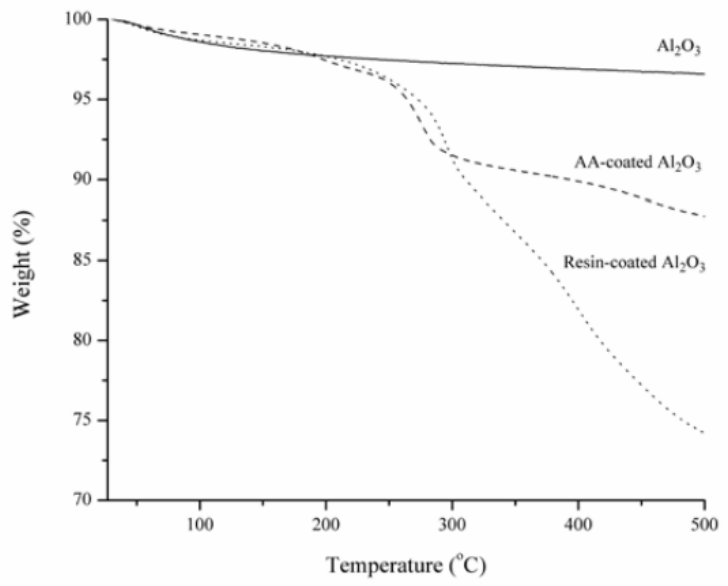


Fig. 2.2. TGA of surface-coated and non-coated particles

Figure 2.3 shows a set of FT-IR spectra for Al_2O_3 (a), AA (b), AA-coated Al_2O_3 (c), DAHA (d), and antibacterial resin-coated Al_2O_3 (e). Spectrum a (alumina) shows peaks at 3415 and 1643 for hydroxyl groups from adsorbed water on alumina particles [25]. It has been reported that this type of water is generally present on any manufactured ceramic particles and difficult to exclude [26].Spectrum b (acrylic acid) shows a broad peak between 3600 and 2400 for carboxyl group (-COOH), a strong peak at 1724 for carbonyl group and two peaks at 1636 and 1618 for carbon-carbon double bonds. In comparison with spectra a, b and c (AA-coated alumina), the appearance of strong peaks at 1728 for carbonyl group and at 1654 for carbon-carbon double bond on spectrum c confirmed successful coating of AA on alumina surface. Furthermore, lack of broad carboxyl peak in spectrum c is also consistent with carboxylic groups intra-structurally linked to alumina particles. Spectrum d

(DAHA) shows strong peaks at 1790 for intra ester group on DA, at 1727 for ester group on HA, and 1626 (small) and 1639 (large) for carbon-carbon double bonds. In comparison with spectra c, d and e, except for the peaks at 3415 and 1643 for hydroxyl groups from adsorbed water on alumina particles (spectrum a and c), the appearance of a peak at 1790 for an intra-ester group on DA, and a peak at 1727 for an ester group on HA on spectrum e confirmed that both DA and HA were successfully coated on AA-coated alumina particle surfaces.

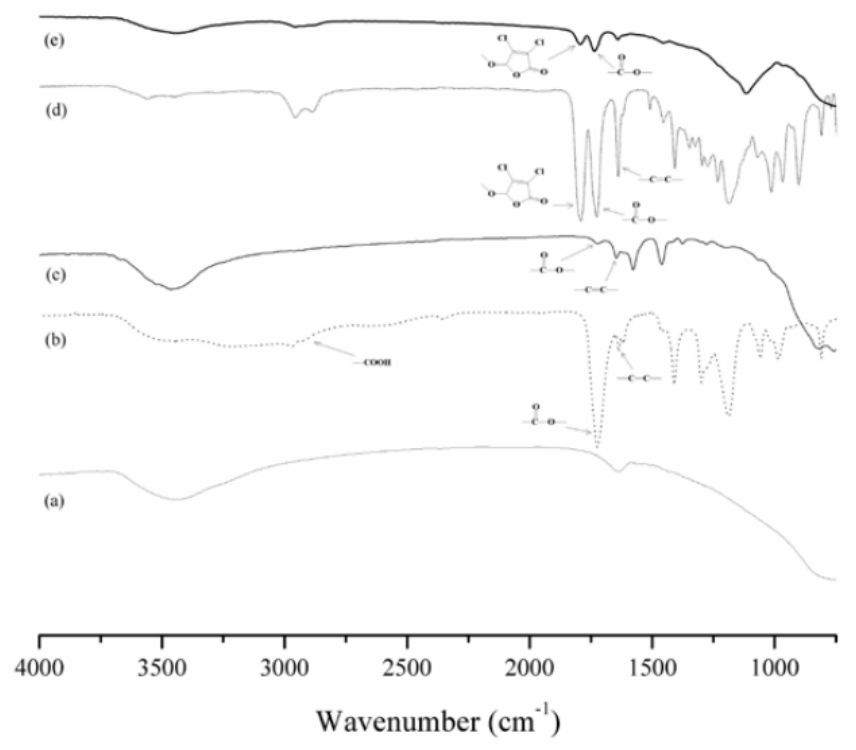


Fig. 2.3. FT-IR spectra of surface-coated and non-coated particles

Figure 2.4 shows a photograph describing the dispersion characteristics of unmodified and modified alumina particles in water and monomer. The unmodified alumina particles (left) were found to be well-dispersed in water layer but not in methyl methacrylate layer at all due to the hydroxyl groups on alumina particle surfaces. On the other hand, either AA-coated (middle) or antibacterial resin-coated alumina particles (right) were found to be well-dispersed in organic methyl

methacrylate layer but not in water layer at all. This can be attributed to the hydrophobic nature of acrylate groups and/or cross-linked hydrophobic antibacterial resin coatings on the modified alumina particles. This photograph also indicates that surface coating was successful.

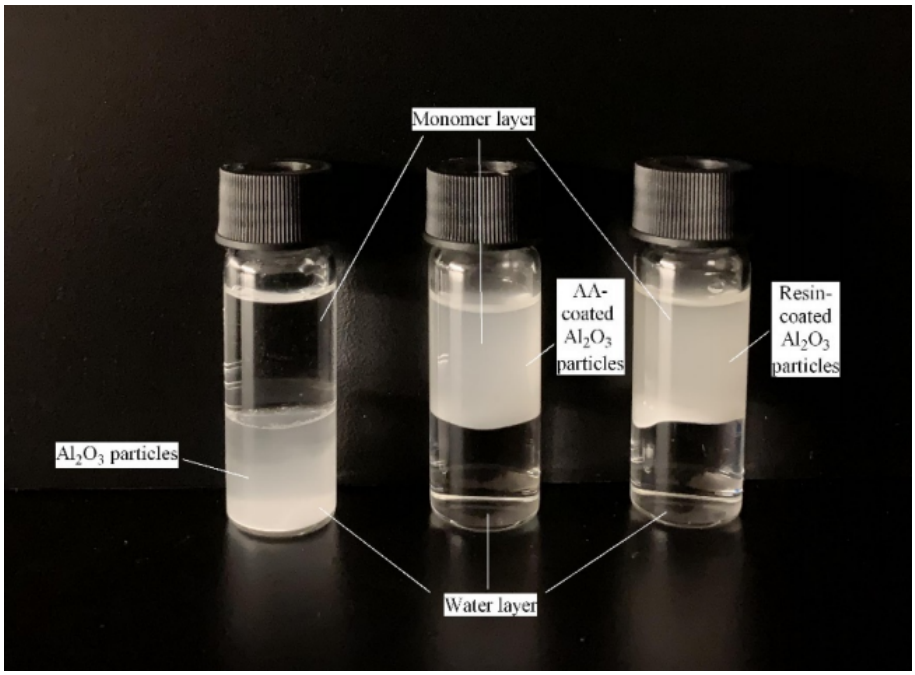


Fig. 2.4. Photograph describing non-coated, AA-coated and antibacterial resin-coated alumina particles in water and methyl methacrylate monomer

2.3.2 Evaluation

Figure 2.5 shows the effects of antibacterial moiety content on Knoop hardness number (KHN) and S. mutans viability. The mean KHN was in the decreasing order of 30% = 25% > 20% > 15% > 10% = 5% > Kerr, where no significant differences were found between 5% and 10% and between 25% and 30% ($p > 0.05$). The mean S. mutans viability was in the decreasing order of Kerr > 5% > 10% > 15% > 20% > 25% > 30%. Apparently, increasing antibacterial moiety content significantly increased KHN and reduced bacterial viability or in other words increased antibacterial activity.

Regarding KHN, antibacterial resin-coated alumina particles increase KHN, which may partially be attributed to stiffer and harder DAHA ring structure (see Figure 2.1). On the other hand, addition of DAHA moiety does exhibit a significantly strong antibacterial function.

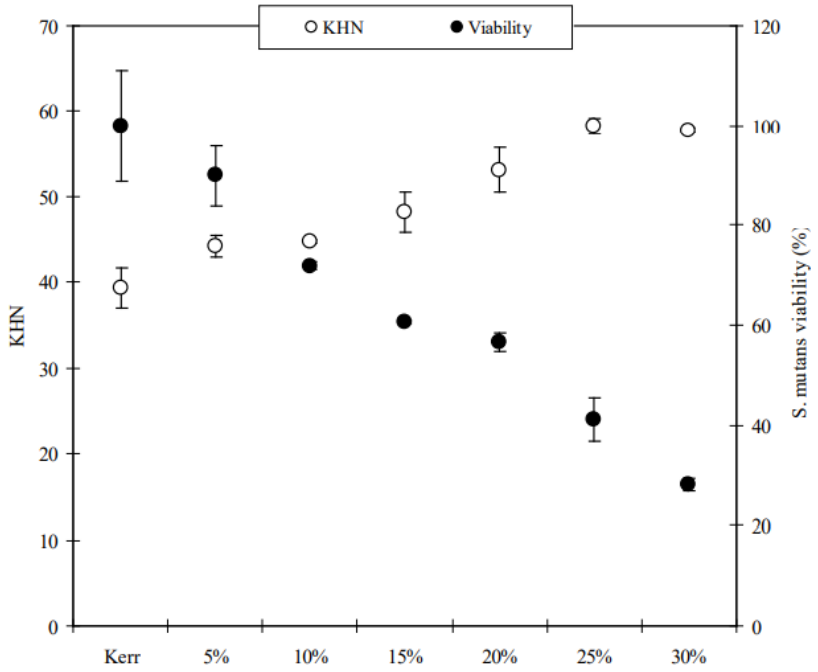


Fig. 2.5. Effect of DAHA moiety content on KHN and S. mutans viability

Figure 2.6 shows the effects of modified alumina particle size on KHN and S. mutans viability. The mean KHN was in the decreasing order of 162 μm > 94 μm = 63 μm > 10 μm > Kerr, where no significant differences were found between 94 μm and 63 μm and between 10 μm and Kerr. The mean S. mutans viability was in the decreasing order of Kerr > 162 μm > 94 μm > 63 μm = 10 μm, where no significant difference was found between 10 μm and 63 μm. It appears that increasing antibacterial alumina particle size slightly increased KHN but did not significantly affect bacterial viability, although the incorporated antibacterial alumina fillers did significantly improve the antibacterial activity as compared to Kerr. The largest

particles (162 µm) showed the 2nd highest bacterial viability to Kerr, indicating that under the same weight smaller particles can deliver more antibacterial residues to the composite due to their larger surface areas, as compared to larger ones.

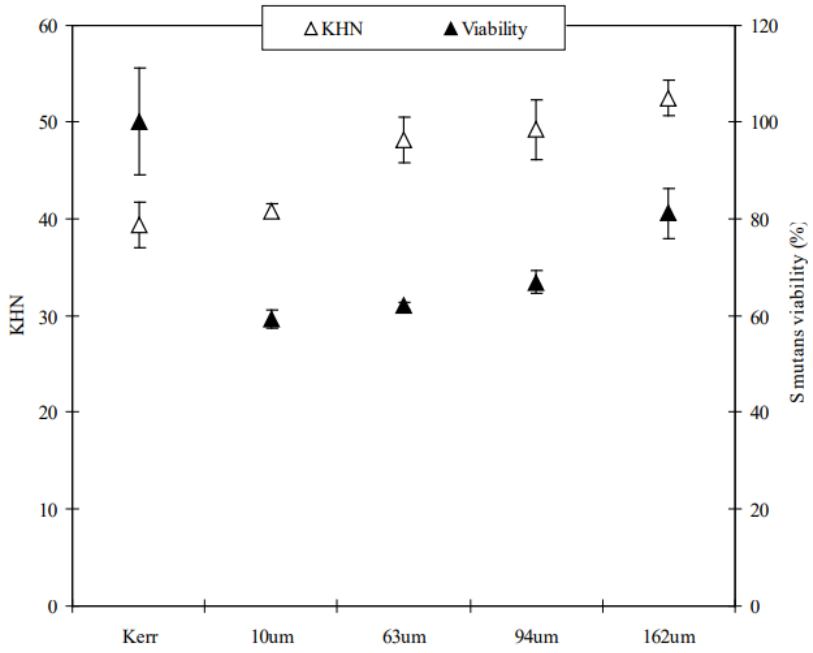


Fig. 2.6. Effect of antibacterial resin-coated particle size on KHN and S. mutans viability

Figure 2.7 shows the effects of antibacterial alumina filler loading on KHN and S. mutans viability. The mean KHN was in the decreasing order of 15% = 20% = 25% > 10% > Kerr, where no significant difference was found among 15%, 20% and 25%. The mean S. mutans viability was in the decreasing order of Kerr > 10% > 15% > 20% > 25%. Increasing antibacterial filler loading increased KHN up to 15% and then nearly no change is observed. On the other hand, increasing filler loading decreased bacterial viability. This can be easily explained as that at the same particle size increasing antibacterial filler loading increases antibacterial moiety contents, thus enhancing the antibacterial activity of the composite.

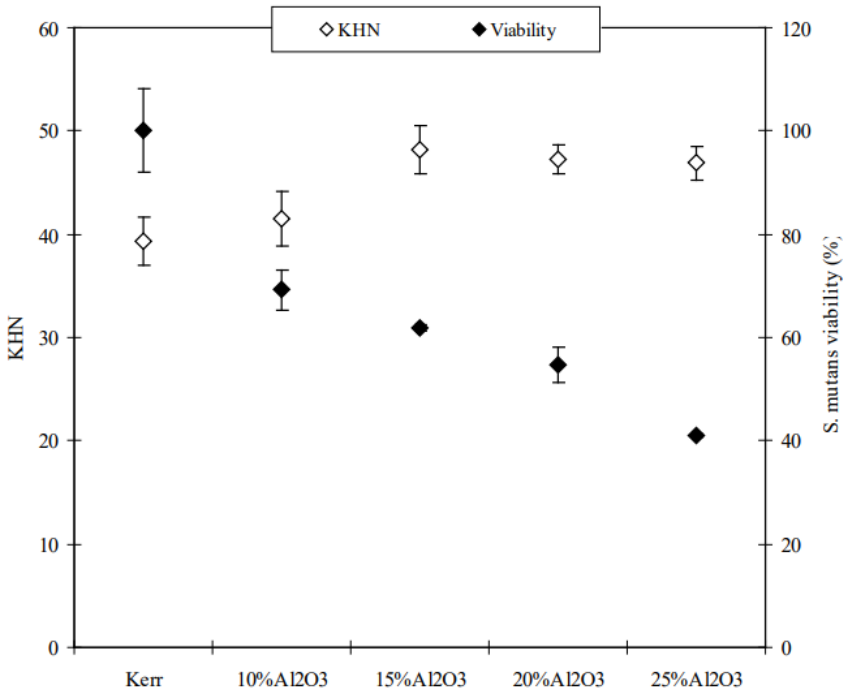


Fig. 2.7. Effect of antibacterial resin-coated filler content on KHN and S. mutans viability

Figure 2.8 shows the effects of total filler content on KHN and S. mutans viability. The mean KHN was in the decreasing order of 79% > 77% > 75% > 73% by weight. The mean S. mutans viability was in the decreasing order of 73% > 75% > 77% > 79%. Increasing total filler content significantly increased KHN but reduced bacterial viability. In composite formulations, total filler content determines hardness and other properties [6, 21]. The higher the filler loading, the higher the hardness the composites are anticipated [6, 21]. Highly filled composites often show the properties that are closer to natural teeth, because teeth are highly mineralized tissue [6, 21]. Therefore, higher filler contents are favored in composite formulations. However, the problem that is often faced during composite preparations is hard to incorporate more filler particles into composites due to interfacial incompatibility between inorganic fillers and organic resin [27]. Fortunately, in this study, since we were able to well

coat the alumina fillers with antibacterial resins, the interfacial compatibility was found to be significantly improved. Thus, the total filler loading was significantly increased, resulting in the fact that not only the total filler content was added up to 79% but also more antibacterial fillers were incorporated. That is why higher total filler loading showed increased hardness and antibacterial activity.

Table 2.1 shows the effect of the modified composites with different filler loading on the viability of four bacterial species. From the results, it is clear that increasing total filler loading decreased bacterial viability. Different bacterial species showed different responses to antibacterial composites. From 73

Table 2.1. Effect of antibacterial fillers on viability of four bacteria (%)[1]

	S. mutans	**S. aureus**	**P. aeruginosa**	**E.Coli**
73%	69.9 (2.7)	63.1 (6.8)	73.2 (6.4)	67.4 (4.4)
75%	49.2 (2.8)	58.9 (1.8)	66.9 (3.1)	41.7 (2.9)
77%	31.0 (3.8)	40.6 (2.8)	37.7 (3.0)	31.9 (2.7)
79%	19.8 (2.8)	23.8 (2.3)	18.9 (0.9)	13.5 (1.4)

[1]Specimens were cultured with bacteria for 48 h before testing

Figure 2.9 shows a set of photomicrographs of bacterial viability after incubating bacteria with the composites, with green fluorescence indicating live bacteria in the culture and red fluorescence indicating dead bacteria. The images depict (a) S. mutans with Kerr (live), (b) S. mutans with Kerr (dead), (c) S. mutans with experimental antibacterial composite (live), (d) S. mutans with experimental composite (dead), (e) S. aureus with experimental composite (live), (f) S. aureus with experimental composite (dead), (g) E. coli with experimental composite (live), and (h) E. coli with experimental composite (dead). Fig 2.9 (a) shows many green (live) bacteria but (b) shows no red (dead) bacteria. In contrast, Fig 2.9 (c) shows significantly lower number of live bacteria whereas (d) exhibits dead bacteria. Fig 2.9 (e) and (g) show

lower number of live S. aureus and E. coli but (f) and (h) exhibit dead S. aureus and E. coli. Apparently, antibacterial composite showed significant antibacterial activity by not only inhibiting bacterial growth but also killing bacteria.

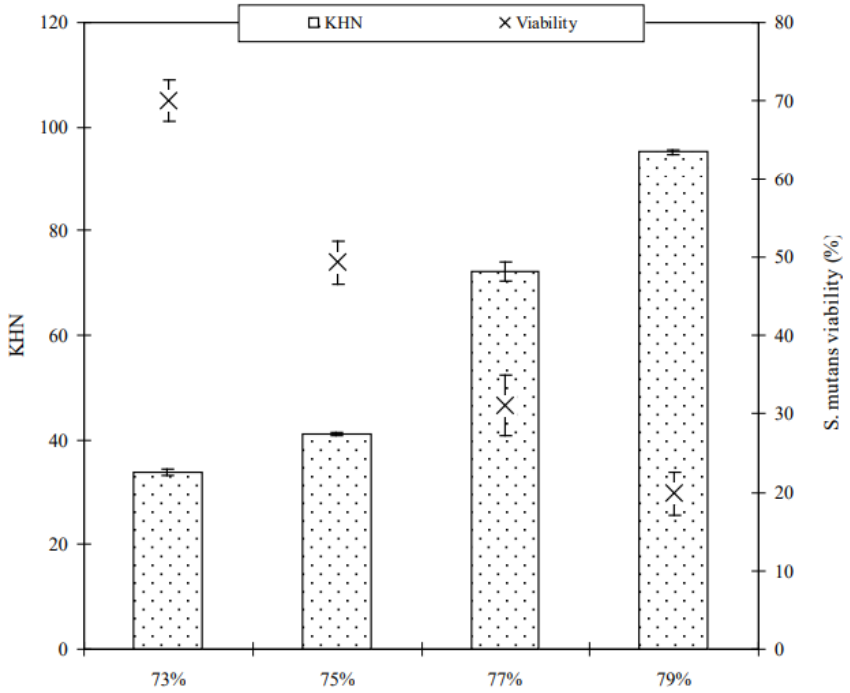


Fig. 2.8. Effect of total filler content on KHN and S. mutans viability

Table 2.2 shows the results from the leaching tests. To test whether antibacterial components from the experimental antibacterial composite would leach out, three experiments - aging, extractable to S. mutans viability and extractable to 3T3 cell viability, were conducted. Theoretically speaking, if there were no changes in bacterial viability during specimen aging, it means that the composite would have no leaching. If there were no changes in bacterial number after S. mutans were cultured with elute, it means that the composite would have no leaching.

Regarding 3T3 fibroblasts, if there were no changes in cell viability after culturing with elute from the composite, it means that the composite would have no leaching. From Table 2.2, it is clear that no significant change in bacterial viability was found

between 1 day and 7 day aging in bacterial broth. Furthermore, no significant change in bacterial number between the antibacterial composite and Kerr was found after culturing with the 48-h elute. For 3T3 viability test, no significant difference in 3T3 viability was found between Kerr and the experimental composite. The results indicate that the experimental composite is a non-leaching antibacterial composite, which eliminates the concern on potential cytotoxicity due to attached antibacterial residues. It also confirms that this novel composite inhibits or kills bacteria by contact but not by the released leachable.

Table 2.2. Effect of antibacterial fillers on viability of four bacteria (%)[1]

	Bacterial viability test (%)$_1$		**Leaching test$_2$**	**Leaching test %$_3$)**
	1 d	7 d	48 h-elute	48-h elute
Kerr	100(1.4)	100(1.4)	5.49 x 10^5/cm_2 (0.31) $_b$	85.7 (5.0)$_c$
EXP	45.0(3.7)^{a,4}	43.1(4.0)^a	5.91 x (10) ^{5}cm^2(0.23)^b	83.6(7.1)^c

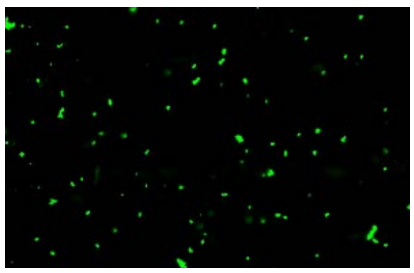
[1]Specimens were cultured with S. mutans for 48 h before testing. [2]Specimens were immersed in bacterial broth at 37 °C for 48 h and then eluate was cultured with S. mutans for 48 h before testing. [3]Specimens were immersed in DMEM at 37 oC for 48 h and then eluate was cultured with 3T3 cells for 48 h before testing. [4]Entries are mean values with standard deviations in parentheses and the mean values with the same superscript letter were not significantly different (p >0.05).

2.3.3 Conclusions

In this study, a novel antimicrobial dental composite has been developed and evaluated. Alumina filler particles were covalently coated with antibacterial resin and blended into a composite formulation. Results showed that almost all the modified composites exhibited higher antibacterial activity along with improved

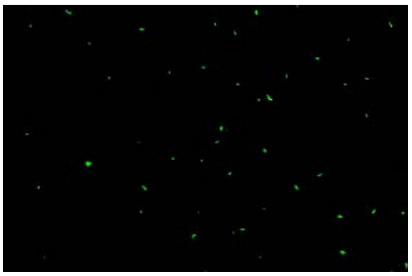
surface hardness, as compared to the unmodified one. Increasing antibacterial moiety content of the added fillers, particle size and loading of the modified fillers, and total filler content generally increased surface hardness.

Increasing antibacterial moiety, filler loading, and total filler content increased antibacterial activity. On the other hand, increasing particle size showed a negative impact on antibacterial activity. The leaching tests indicate that the experimental antibacterial composite showed no leachable antibacterial component to bacteria and 3T3 mouse fibroblasts. Future studies will include formulation optimization and other mechanical and physical property evaluations.

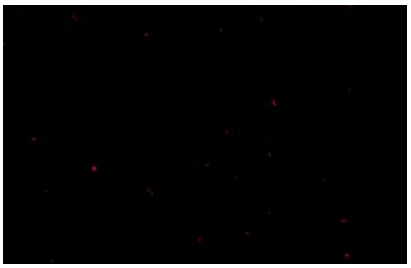


(a) S. mutans with Kerr (live)

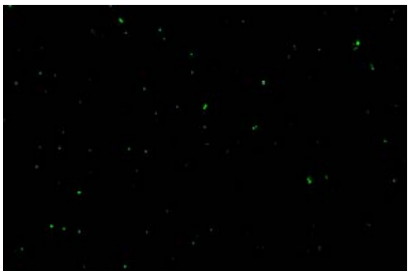
(b) S. mutans with Kerr (dead)



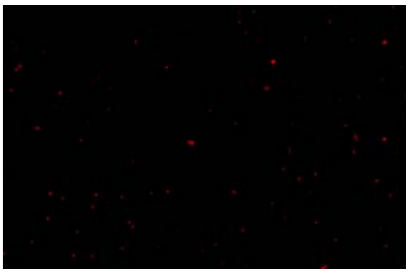
(c) S. mutans with antibacterial composite (live)



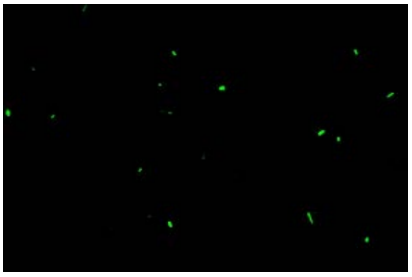
(d) S. mutans with antibacterial composite (dead)



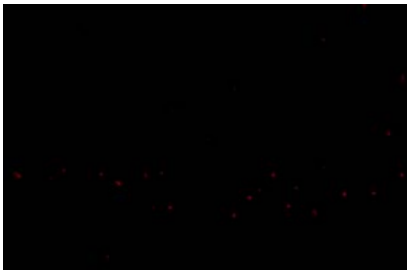
(e) S. aureus with antibacterial composite (live)



(f) S. aureus with antibacterial composite (dead)



(g) E. coli with antibacterial composite (live)



(h) E. coli with antibacterial composite (dead)

Fig. 2.9. Bacterial images after incubating with antibacterial composite vs. Kerr for 48 h. Bacteria were stained with a fluorescence viability stain, with green fluorescence indicating live cells in the culture and red fluorescence indicating dead cells

CHAPTER 3. COATING OF ZIRCONIA FILLERS FOR IMPROVED ANTIBACTERIAL DENTAL PROPERTY

3.1 Introduction

Antibacterial biomedical materials or devices are attractive to medical fields, with no exception to dental restorations. Clinically attractive dental filling restoratives are supposed to prevent tooth from forming secondary caries [1, 4]. Secondary caries is a type of caries that is generated by oral bacteria after dental restorations. It is a tooth demineralization produced by plaque bacteria such as Streptococcus mutans in the presence of fermentable carbohydrates. The process°Ccurs at the interface between the cavity preparation and the restoration [3]. Secondary caries has been reported to be the main reason to the dental restoration failure [1, 4]. Therefore, secondary caries prevention is very important to restorative dentistry. There have been two main strategies to prevent secondary caries caused by oral bacteria. The first one is to incorporate low molecular weight antibacterial drugs into dental restorative formulations, where the mechanism is based upon release or slow-release of these low molecular weight drugs. Such drugs include but are not limited to various antibiotics, chlorhexidine, zinc ion, silver ion and iodine [5, 7]. However, release or slow-release of these drugs can suffer from a mechanical property reduction of the restoratives over time, have short-term effectiveness but long-term run-out of the releasing drugs, produce possible toxicity of the drugs to surrounding tissues, and enhance a chance for antibiotic-resistant bacteria formation [5, 7]. The second one is to incorporate high molecular weight antibacterial polymers or covalently link antibacterial drugs to restoratives or devices [19, 28], where the mechanism is based on "kill by contact" [9]. The second strategy is considered to be more effective than the first one. One of the typical examples is to

incorporate organic quaternary ammonium salts into the restoratives [10, 28]. 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 [11]. There are numerous examples of using the quaternary ammonium salt derivatives for dental restoratives include applying polymerizable methacryloyloxydodecyl pyridinium bromide in composites [29], adding polyethylenimine quaternary ammonium nanoparticles to composites [14], and incorporating polymerizable quaternary ammonium bromide derivatives with different chain lengths into glass-ionomer cements [10]. The results showed that all the above quaternary ammonium salt-modified dental restoratives did exhibit significant antibacterial activities. Another example of using the second strategy is to incorporate furanone-derivatized drugs into restoratives. The furanone derivatives have shown strong antitumor [15, 16] and antibacterial functions [17]. Recently these derivatives were successfully incorporated into dental glass-ionomer cements [18] and dental composites [19],, resulting in the promising outcomes [18, 19]. 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 [20]. Therefore, in this study, we proposed to use zirconia particles as a delivery vehicle to deliver antibacterial drug by covalently coating a cured antibacterial drug-containing polymer on zirconia particle surfaces, followed by thoroughly washing to remove the uncoated derivatives and then incorporating into the system, to reduce the leachable. Zirconia is known to be radio-opaque and biocompatible [27]. Using it as a delivery vehicle would also enhance the radio-opacity of the modified composites.

The purpose of this study was to coat an antibacterial furanone derivative onto zirconia fillers, use these coated particles to formulate an antibacterial dental composite for improved antibacterial activity, and evaluate the compressive strength and antibacterial function of the formed composite.

3.2 Materials and Methods

3.2.1 Materials

Bisphenol A glycidyl methacrylate, triethylene glycol dimethacrylate, acrylic acid, 2-hydroxyethylacrylate,p-toluenesulfonicacidmonohydrate, 2,3-dichloromalealdehydic acid, toluene, sodium bicarbonate, -(trimethoxysilyl)propyl methacrylate, potassium persulfate, camphoroquinone, and 2-(dimethylamino)ethyl methacrylate, and zirconia fillers (amorphous) were received from Sigma-Aldrich Co. (Milwaukee, WI) and used without further purifications. The Herculite-XRV (particle = 0.7 microns, untreated) glass fillers were received as a gift from Kavo Kerr Dental Specialties (Orange, CA).

3.2.2 Synthesis and characterization

3.2.2.1 Synthesis of 2,3-dichloro malealdehydic acid hydroxyethylacrylate

To a solution containing 3,4-dichloromalealdehydic acid (0.1 mol), toluene and p-toluene sulfonic acid monohydrate (1% by mole), 2-hydroxyethyl acrylate (0.11 mol) in toluene was added [8]. After the mixture was run at 90-100°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. The synthesis scheme is shown in Figure 3.1.

3.2.2.2 Surface coating of antibacterial resin onto zirconia fillers

Surface coating was accomplished with the following three steps: (1) Surface activation with acrylic acid. Zirconia particles were dispersed in acrylic acid with ultrasonic vibration for 10 min [23], followed by heating at 70°C overnight, washing with methanol and filtering. (2) Fixation of antibacterial agent on particle surface. This process was conducted by immersing acrylic acid-activated zirconia particles in a mixture of DH and triethylene glycol dimethacrylate in methanol, followed

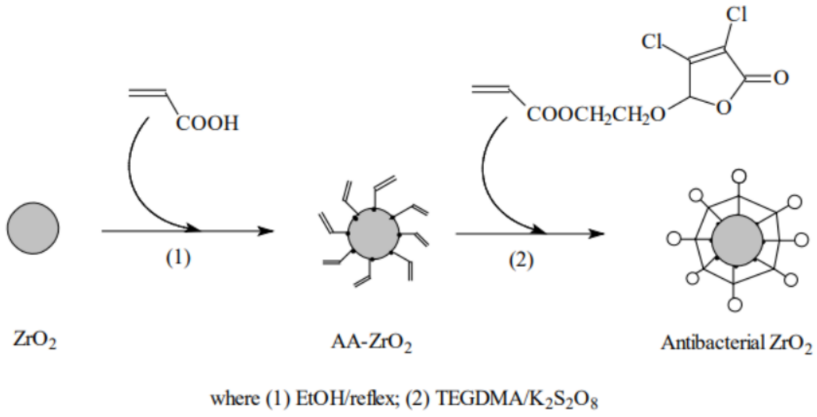


Fig. 3.1. Schematic diagrams for preparation of antibacterial resin-coated zirconia particles

by removing methanol with a rotary evaporator. (3) Covalently coating DH and crosslinking with triethylene glycol dimethacrylate on the particle surfaces. This process was completed by dispersing the particles in distilled water containing potassium persulfate, followed by heating at 70°C for 3 h, filtering and freeze-drying. The coating scheme is shown in Figure 3.1.

3.2.2.3 Characterization

The zirconia particle 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).

3.2.3 Evaluation

3.2.3.1 Specimen preparation for evaluations

The experimental composites were formulated with a two-component (powder and liquid) system [20]. The glass powders (silicon dioxide, Herculite XRV) were

treated with -(trimethoxysilyl)propyl methacrylate, following the published protocol [20]. The treated glass powders were then blended with the antibacterial resin-coated zirconia particles using a vortex mixer. The liquid portion was formulated with bisphenol A glycidyl methacrylate (BisGMA, 50% wt/wt), triethylene glycol dimethacrylate (TEGDMA, 50%), camphoroquinone (photo initiator, 1%), and 2-(dimethylamino)ethyl methacrylate (activator, 2%), following the protocol elsewhere [20]. A glass filler content at 75% (wt/wt) or glass filler powder/resin liquid (P/L) ratio at 3/1 was applied throughout the study unless specified.

Specimens were prepared by mixing the liquid with the glass fillers thoroughly at room temperature, according to the published protocol [20]. Briefly, the cylindrical specimens 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 specimens 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.

3.2.3.2 Compression test

CS 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 [13]. CS was calculated using an equation $P/^2$, 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.

3.2.3.3 Bacterial Viability Test

The bacterial viability test was carried out based on the protocol described elsewhere [8]. Bacterial colonies were suspended in 5 mL of tryptic soy broth, supplemented with 1% sucrose, to form a suspension with 108 CFU/mL of bacteria

and incubated for 24 h. Four bacterial species including Streptococcus mutans (S. mutans), Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa) and Escherichia coli (E. coli) 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 fluorescent green/red (1:1 v/v) stain (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 the 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 bacterial suspension without disks was used as control and viable bacteria counts from the suspension were used as 100%. The viability was analyzed by counting from the recorded images with using ImageG software. Triplicate samples were used to obtain a mean value for each material in each test.

3.2.3.4 Statistical Analysis

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

3.3 Results and Discussion

3.3.1 Characterization

Figure 3.2 shows a set of FT-IR spectra for ZrO2 (a), acrylic acid-coated ZrO2 (b) and resin-coated ZrO2 (c). Spectrum a shows peaks at 3418 cm-1 hydroxyl groups from adsorbed water on zirconia particles [30]. It has been reported that this type

of water on any manufactured ceramic particles are hardly removed [26]. 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 zirconia 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 zirconia particle surfaces.

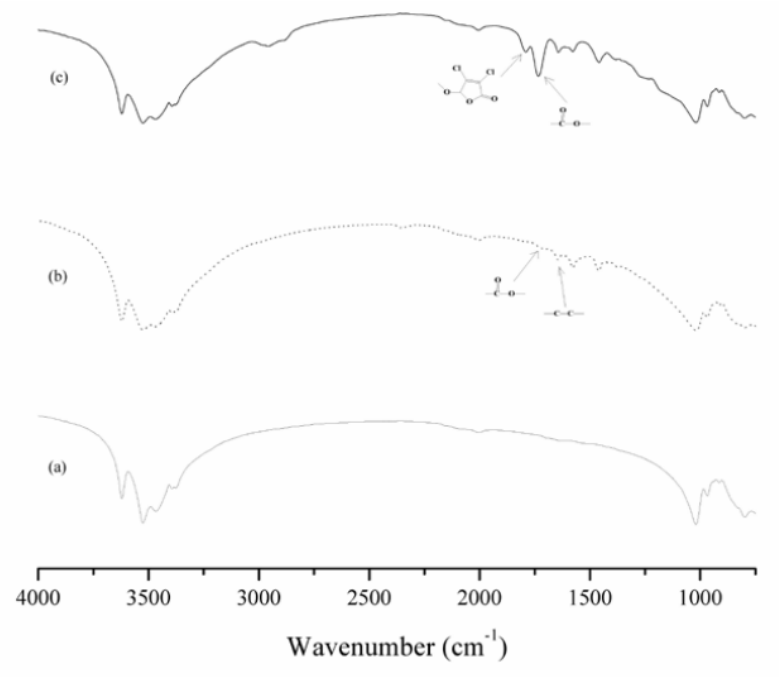


Fig. 3.2. FT-IR spectra of surface-coated and non-coated particles

3.3.2 Evaluation

Figure 3.3 shows the effects of antibacterial moiety content on CS and S. mutans viability. The mean CS (MPa) was in the decreasing order of control > 15% = 10% = 5%, where no significant differences were found among them ($p > 0.05$). The mean

S. mutans viability was in the decreasing order of control > 5% > 10% > 15%. With increasing antibacterial moiety content, CS showed nearly no change, but bacterial viability was significantly decreased, or antibacterial activity was increased. Since only 7% antibacterial resin-coated zirconia was added, CS did not show statistically significant changes. On the other hand, addition of DH moiety does exhibit a significantly strong antibacterial function.

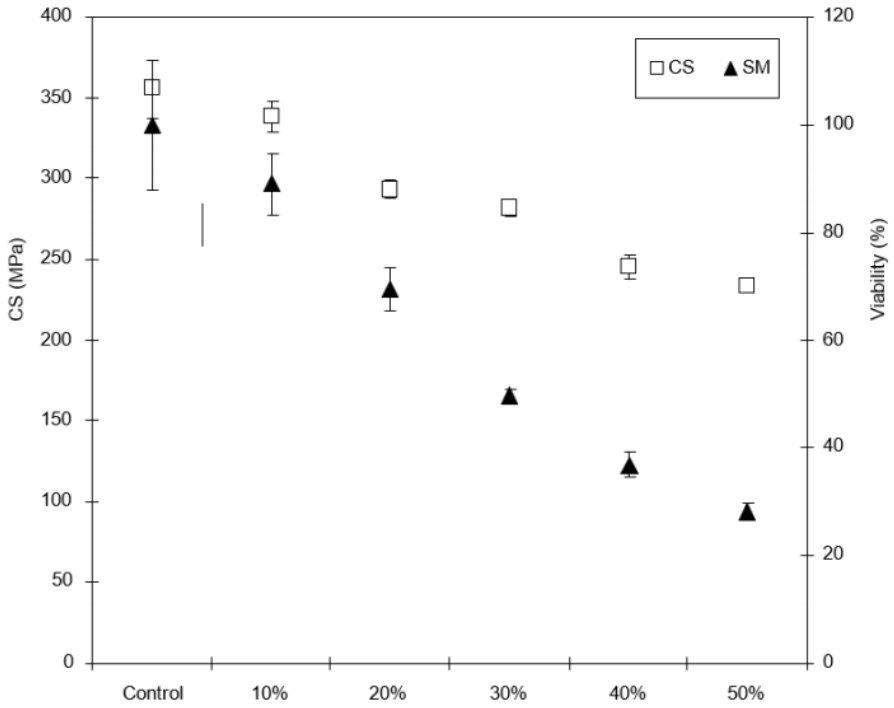


Fig. 3.3. Effect of DH moiety content on CS and S. mutans viability

Figure 3.4 shows the effects of antibacterial zirconia filler loading on CS and S. mutans viability. The mean CS was in the decreasing order of control > 10% > 20% > 30% > 40% > 50%, where no significant difference was found between control and 10%. The mean S. mutans viability was in the decreasing order of control > 10% > 20% > 30% > 40% > 50%. With increasing antibacterial filler loading, CS was significantly decreased. This is probably because the added zirconia fillers are amorphous, which did not provide any strength enhancement function to the

system. On the other hand, the bacterial viability was significantly decreased with increasing filler loading. This can be easily explained as that increasing antibacterial filler loading increases antibacterial moiety contents, thus enhancing the antibacterial activity of the composite.

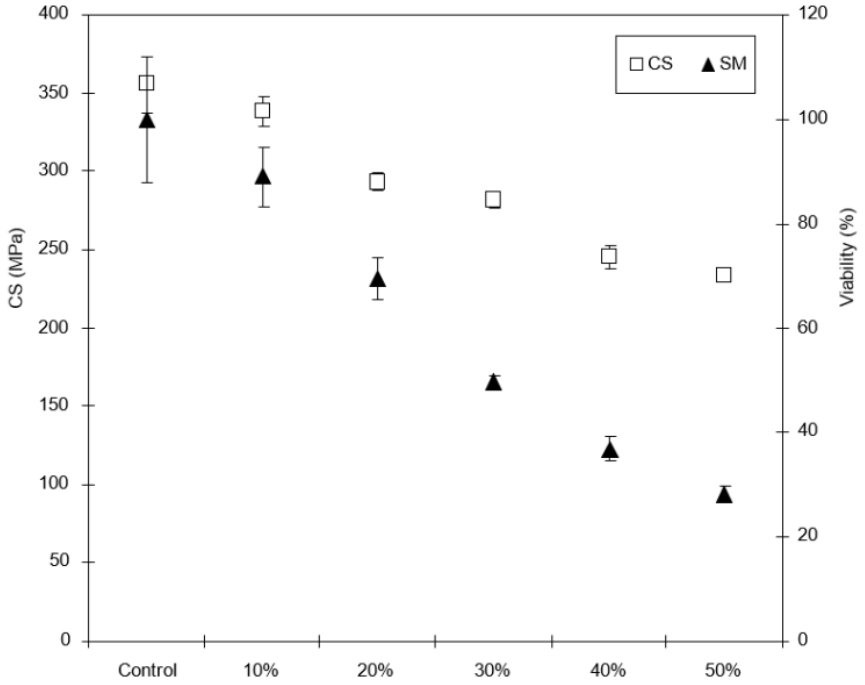


Fig. 3.4. Effect of antibacterial resin-coated filler content on CS and S. mutans viability

Figure 3.5 shows the effects of P/L ratio on CS and S. mutans viability. The mean CS was in the decreasing order of control $>$ 3.6 $>$ 3.3 $=$ 3.0 $>$ 2.7 by weight, where no significant difference was found between 3.3 and 3.0. The mean S. mutans viability was in the decreasing order of control $>$ 2.7 $>$ 3.0 $>$ 3.3 $>$ 3.6. Clearly with increasing P/L ratio CS was significantly increased with the lowest at 2.7 but the highest at 3.6 but the highest value was still lower than control. On the other hand, bacterial viability was significantly decreased. It is known that glass fillers are inorganic components which often enhance compressive strengths, but organic resins often enhance plastic properties or reduce compressive strength [6, 31].

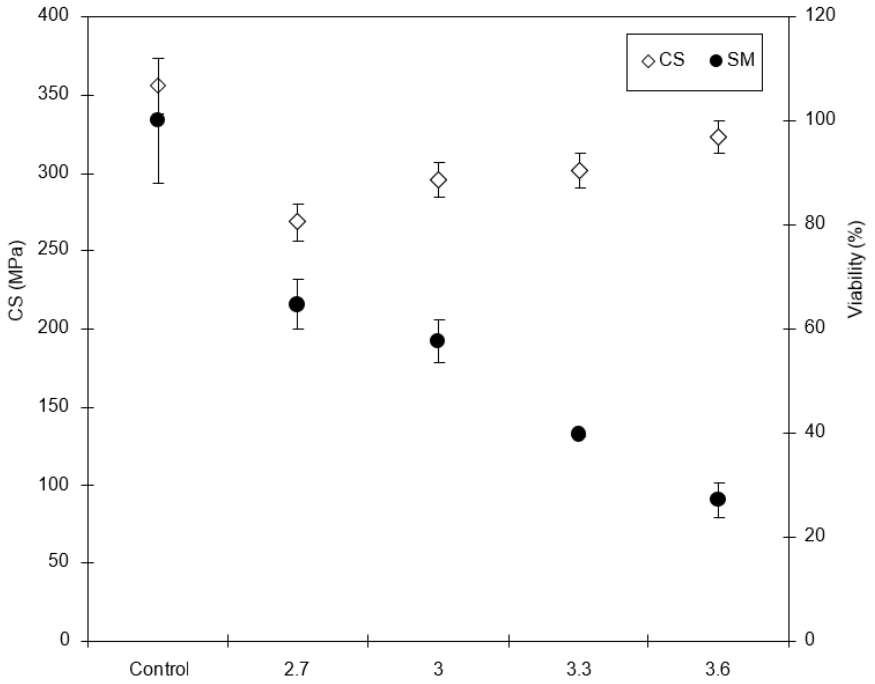


Fig. 3.5. Effect of P/L ratio on CS and S. mutans viability

Since the added zirconia 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 P/L ratio or total filler loading, the corresponding coated antibacterial moiety content was also increased, thus resulting in an enhanced antibacterial activity.

Table 3.1 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 control showed significantly higher values than all the modified composites, but no significant differences were generally found among the modified composites. For antibacterial filler loading, increasing filler loading significantly decreased YS, M and CS, where control showed significantly higher values than all the modified composites and the composite with 50% loading showed the lowest values. The reason was the same as discussed previously. For

Table 3.1. Effects of zirconia loading and P/L ratios on CS. [1]Specimens were conditioned in distilled water at 37 °C before testing. [2]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)

	YS (MPa)	Modulus (GPa)	CS (MPa)
	Effect of DH moiety content		
Control	149.8 (5.2)	6.90 (0.08)	355.6 (18)
5%DH	145.3 (4.8)	6.37 (0.06)	336.5 (7.0)
10%DH	140.6 (0.9)	6.31 (0.03)	324.4 (5.7)
15%DH	139.3 (5.2)	6.22 (0.17)	328.8 (10)
	Effect of antibacterial resin-coated filler content		
10%ZrO2	144.0 (5.3)	6.31 (0.06)	337.8 (9.2)
20%ZrO2	135.9 (3.6)	5.89 (0.10)	293.4 (5.5)
30%ZrO2	122.9 (3.9)	5.52 (0.08)	281.4 (4.9)
40%ZrO2	119.1 (6.8)	5.41 (0.17)	245.6 (7.8)
50%ZrO2	103.8 (5.0)	5.02 (0.03)	233.3 (4.0)
	Effect of P/L ratio		
2.7	120.8 (6.4)	5.48 (0.08)	268.3 (11)
3	125.8 (2.4)	5.71 (0.01)	295.5 (11)
3.3	130.4 (4.1)	6.04 (0.12)	301.5 (12)
3.6	142.3 (4.8)	6.42 (0.13)	323.1 (10)

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 [6, 21]. Zirconia 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 zirconia fillers both YS and M were significantly increased.

Table 3.2. Effect of P/L ratio on viability of four bacteria (%). Specimens werecultured with bacteria for 48 h before testing

	S. mutans	**E.Coli**	**S. aureus**	**P. aeruginosa**
Control	100 (12.1)	100 (1.4)	100(10.7)	100 (9.3)
2.7	64.7(4.7)	76.3(5.9)	79.5(3.2)	85.7(5.5)
3.0	57.6(4.1)	65.2(1.7)	64.9(3.6)	68.9(5.4)
3.3	27.1(3.4)	15.1(0.5)	22.9(0.7)	30.9(0.8)

Table 3.2 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 composites. From 2.7 to 3.6, S. mutans, E. coli, S. aureus and P. aeruginosa showed 58%, 80%, 71% and 64% decrease in bacterial viability, respectively. As compared with control, S. mutans, E. coli, S. aureus and P. aeruginosa 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. S. mutans, E. coli and S. aureus are more vulnerable to the antibacterial compound DH than P. aeruginosa.

3.4 Conclusion

An antibacterial resin was covalently coated onto the surface of zirconia filler particles. The coated fillers were incorporated into a dental composite. Compressive strength and bacterial viability were used to evaluate the modified composites. Results showed that almost all the modified composites exhibited higher antibacterial activity along with decreased compressive strength, as compared to the unmodified control. With increasing antibacterial moiety 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 moiety content, filler loading and powder/liquid ratio, antibacterial activity was enhanced.

CHAPTER 4. CONCLUSIONS

We have developed a novel antibacterial dental composite restorative system. Results showed that almost all the modified composites exhibited significant higher antibacterial activity than the unmodified one. For alumina-based antibacterial filler modification, increasing antibacterial moiety content, particle size and loading, and total filler content generally increased surface hardness. Increasing antibacterial moiety, filler loading, and total filler content increased antibacterial activity. On the other hand, increasing particle size showed a negative impact on antibacterial activity. The leaching tests indicate that the experimental antibacterial composite showed no leachable antibacterial component to bacteria and 3T3 mouse fibroblasts. For zirconia based antibacterial filler modification, increasing antibacterial moiety content and modified filler loading, yield strength, modulus and compressive strength of the composite were decreased. Furthermore, the strengths of the composite were increased with increasing powder/liquid ratio. On the other hand, with increasing antibacterial moiety content, filler loading and powder/liquid ratio, antibacterial activity was enhanced. Future study will include formulation optimization and other mechanical and physical property evaluations.

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