ORIGINAL ARTICLE

Evaluation of antimicrobial properties of orthodontic composite resins combined with benzalkonium chloride

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An antimicrobial agent, benzalkonium chloride (BAC), was added to a chemically cured composite resin, and the antimicrobial benefits and physical properties of the modified composite were evaluated. BAC was added to Reliance Phase II composite to create modified composites with BAC concentrations ranging from 0.25 to 2.50 wt%. Specimen disks of the modified composite were incubated with *Streptococcus mutans* for 48 hours, and an agar disk diffusion assay was used to measure zones of bacterial inhibition. Larger disks were suspended in brain-heart infusion medium containing 2×10^4 colony-forming units/mL *Streptococcus sobrinus* (10 mL, 2 wt% sucrose, 24 hours) to measure bacterial adherence to the adhesive; adherent cells were removed from the surface with 1 N NaOH, and the optical density of the cells was measured at 550 nanometers. Traction hooks were bonded to bovine teeth with the modified composite, and tensile bond strength was evaluated with a universal testing machine. Diametral tensile stress was also measured. The modified composite samples showed that antimicrobial activity increased with higher BAC content; no antimicrobial activity was measured for the original compound in either the disk diffusion or the bacterial adherence test. There were no significant differences ($P < .05$) in either tensile bond strength or diametral tensile stress among the modified composite groups and the original product. The incorporation of BAC in composite material added antimicrobial properties to the original compound without altering its mechanical properties. (Am J Orthod Dentofacial Orthop 2002;122:288-94)

The quality and ease of handling of dental materials have improved greatly, but little attention has been directed toward increasing their antimicrobial and biologic properties. Fixed orthodontic appliances continue to present oral hygiene problems. A higher incidence of white spot lesions is reported in orthodontic patients than in control populations.[1-6]

Various antimicrobial agents have been incorporated into oral products and approved for intraoral use. Most are designed to prevent plaque accumulation and thereby prevent or treat gingivitis. Chlorhexidine remains the most effective antiplaque and antigingivitis agent.[7] Recently, researchers[8-10] modified filling materials by adding antimicrobial agents such as chlorhexidine and quaternary ammonium compounds to composite resins, acrylic resins, and glass ionomer cements. They found that these agents, added in minute amounts, could impart an antibacterial trait to dental materials without significantly affecting their physical properties.

No orthodontic adhesive containing an antimicrobial agent is commercially available, despite the need for a material that combats the microbial attack on the adhesive[11] and the tooth structure.

In a clinical trial that examined the effect of fluoride-releasing bonding systems, it was found that the addition of fluoride did not significantly reduce the incidence of enamel decalcification; 50% of the patients and 13.5% of the teeth had posttreatment decalcification.[12] In similar clinical trials, there was no significant difference between the decalcification rates when a visible light-cured fluoride-releasing material was compared with a chemically cured nonfluoride resin during fixed orthodontic therapy,[13] or when the Fuji glass ionomer bonding system (GC America Inc, Alsip, Ill) was compared with a light-cured composite resin (Re-

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Submitted, July 2001; revised and accepted, November 2001.

0889-5406/2002/$35.00 + 0 **8/1/123947**
doi:10.1067/mod.2002.123947

liance Light Bond; Reliance Orthodontic Products, Itasca, Ill).[14]

To be accepted clinically, modified materials must provide superior antimicrobial activity and display comparable physical properties such as tensile and shear bond strength, when compared with conventional adhesives. Fluoride is the most common preventive additive in orthodontic adhesives. Conventional glass ionomer cements have very low shear bond strength and are not appropriate for routine orthodontic bonding.[15] A resin-reinforced glass ionomer cement, Fuji Ortho (GC America Inc), has been introduced for orthodontic bonding procedures to overcome the low shear strength of conventional glass ionomer cements. The tensile and shear bond strengths of Fuji Ortho were found to be significantly higher than those of conventional glass ionomer cements[16]; however, when compared with a light-cured composite, Reliance Light Bond, Fuji Ortho had a higher failure rate.[14]

We propose that adding a minute amount of an antimicrobial agent to a commercially available composite resin will impart antimicrobial qualities without significantly altering the physical properties of the compound.

MATERIAL AND METHODS

Reliance Phase II chemical cure composite (Reliance Orthodontic Products), called the original composite material, was used in this study. It was modified with the antimicrobial agent benzalkonium chloride (BAC) (ICN Biomedicals Inc, Aurora, Ohio) in either pure (powder) form or 50% (aqueous) form.

Pure BAC initially was diluted to 25% by mixing it with part A of the Phase II primer. Part A primer was added to part A of the original composite that was later mixed with part B composite according to the manufacturer's instructions. The final BAC concentrations in the original composites were 0.25%, 0.75%, 1.25%, 1.75%, and 2.5% (wt/wt); the modified original composite material is called the BAC (pure)-composite. The BAC (aqueous)-composite was produced by adding 50% BAC to the composite mixture. All BAC-modified composites were made into sample disks of uniform size (5.5 mm diameter $\times$ 3.3 mm thick) with custom-made molds.

Circular traction hooks, 3 mm in diameter (TP Orthodontics, LaPorte, Ind), bonded to bovine teeth were used to measure tensile bond strength with a universal testing machine (Model 1125; Instron, Canton, Mass). Oral cariogenic streptocci, *Streptococcus mutans* 10449 and *Streptococcus sobrinus* B13, were the test bacteria. These were grown routinely in a brain-heart infusion medium (Difco, Detroit, Mich) at 37°C overnight.

Antimicrobial activity of BAC

After the BAC was mixed with part A of the Phase II bonding agent, its antimicrobial activity was tested against *S mutans*. The minimum inhibitory concentration (MIC) was determined against test bacteria in sterile 96-well microtiter plates. Each well contained 5×10^5 colony-forming units/mL of test bacteria, serially diluted BAC, and brain-heart infusion broth. For all MIC testing, triplicate samples were performed for each test concentration. The controls included inoculated broth without BAC. Uninoculated medium served as sample blanks. All plates were incubated aerobically at 37°C in a stationary environment, and growth was estimated spectrophotometrically (660 nanometers [nm]) after 48 hours with a microplate reader (Molecular Device; Vmax Kinetics, Menlo Park, Calif). The MIC for each test bacteria is defined as the minimum concentration of test compound limiting turbidity to < 0.05 absorbance at 550 nm.

Antimicrobial activity of BAC (pure)-composite samples

Disk diffusion assay method. Brain-heart infusion agar plates were inoculated with 80 μL of *S mutans* 10449 from a 60-fold dilution of a 0.5 optical density (550 nm) cell suspension prepared in a 24-hour culture. The inoculum was distributed evenly on the plate surface with a glass rod to provide a uniform bacterial growth. BAC (pure)-composites were placed on the surface of the agar, and the plates were incubated for 48 hours at 37°C. After 48 hours, the inhibition zone around each sample was measured and recorded.

The effect of storage time on the antibacterial activity of the composite samples was evaluated by soaking them in distilled water at 37°C for periods ranging from 10 to 240 days. At the end of each period, the samples were air dried in an aseptic environment for 2 hours and tested as previously described.

Bacterial adherence to BAC (pure)-composite samples

BAC (pure)-composite samples were formed in 9 $\times$ 3 mm custom-made molds. Five samples were made for each concentration (0%, 0.25%, 0.75%, 1.25%, 1.75%, and 2.5%) for a total of 30 samples. The method previously described by Evans et al[17] was used. BAC (pure)-composite samples were suspended on stainless steel orthodontic wires in test tubes containing brain-heart infusion broth culture media enriched with 3% sucrose and incubated for 24 hours at 37°C. After

incubation, the samples were removed and gently rinsed with sterile distilled water to dislodge loosely bound bacteria. The samples were then transferred to 1 mL of 1 N NaOH and sonicated for 10 minutes to dislodge the attached plaque. The absorbance of the bacterial suspensions was measured at 550 nm with a spectrophotometer (Spectronic 601; Milton Roy, Rochester, NY).

BAC incorporation and physical properties of composite

Testing bond strength of BAC (pure)-composite. Tensile bond strength, rather than shear bond strength, was tested because it yields more consistent results and is relatively insensitive to minor misalignment errors.[18] Thirty-two bovine incisors were embedded individually in an acrylic mold with the labial surface parallel to the mold base. BAC was mixed in different amounts with the original composite as described above. The resulting BAC (pure)-composite was used as a bonding agent.

The teeth were prepared for bonding by cleaning the facial surface with a rubber cup and nonfluoride pumice. The bonding surface was etched with 38% phosphoric acid gel (Etch-Rite; Pulpdent Corp, Watertown, Mass) for 20 seconds and rinsed with water for 30 seconds, and the teeth were allowed to air dry for an hour. Traction hooks were bonded on the labial surface of the teeth with composite resin containing various concentrations of the antimicrobial agent. The manufacturer's instructions were followed when bonding the traction hooks to the labial side of each tooth. The teeth were assigned to 1 of 4 groups (8 teeth each) according to the following criteria: group A, traction hooks were bonded with the original adhesive; group B, traction hooks were bonded with adhesive mixed with 0.75% (wt/wt) antimicrobial agent; group C, traction hooks were bonded with adhesive mixed with 1.75% (wt/wt) antimicrobial agent; group D, traction hooks were bonded with adhesive mixed with 2.50% (wt/wt) antimicrobial agent.

The tensile bond strength of each specimen was tested at a crosshead speed of 1 mm per minute. The samples were attached to the lower part of the universal testing machine. The attachments were connected to the upper part with 0.017 × 0.025-in stainless steel orthodontic rectangular wires. Universal joints were used to ensure proper alignment of the samples in the machine. The bond strength (BS) was calculated with the following equation, in which BS = bond strength, r = sample radius, and P = load:

$$BS = P/\pi r^2$$

Testing diametral tensile strength of BAC (pure)-composite. The effect of BAC on the diametral tensile strength of the composite was investigated. Composite specimen disks measuring 5.5 × 3.3 mm and containing 0.00%, 0.25%, 0.75%, 1.25%, 1.75%, and 2.50% BAC were made as described previously. The samples were tested for diameteral tensile strength at a crosshead speed of 2 mm per minute. The diametral tensile strength was determined with the following equation, in which DT = diametral tensile strength, P = load, d = diameter, and t = thickness:

$$DT = 2P/\pi\, d\, t$$

DATA ANALYSIS

The data were analyzed to determine the mean and the SD. One-way analysis of variance (ANOVA) was used to determine whether a significant difference existed between the various groups, and the Scheffé method was used to compare these differences.

RESULTS

BAC was mixed with the part A primer and tested in vitro to determine whether the primer affected its antimicrobial activity against *S mutans.* The MIC value was 0.0015 mg/mL, indicating BAC's continued ability to inhibit growth of *S mutans*; the MIC value remained consistent throughout the 4 trials.

Disk diffusion assay method

The disk diffusion assay method was used to demonstrate the ability of the BAC-modified composite material to release BAC into the surrounding agar media and thereby inhibit growth of *S mutans*. The growth effect was determined by measuring the diameter of the zone of growth inhibition around the BAC (pure)-composite disks. Initially, the experiment was carried out with BAC (aqueous)-composite samples; their antimicrobial activity increased as the concentration of BAC increased. Samples containing 2.50% BAC exhibited the highest antimicrobial activity. When BAC (aqueous)-composites were stored in distilled water, their antimicrobial activity diminished rapidly during the first 30 days but remained measurable beyond day 26 (Fig 1).

The rapid depletion of BAC was minimized with an alternative dilution method in which the pure form of BAC was used to prepare the modified composite. Varying amounts of solid BAC were diluted in part A of the orignal composite material to produce BAC (pure)-composites. The original composite material used as the control demonstrated no inhibition zone when placed on an agar plate preinoculated with *S*

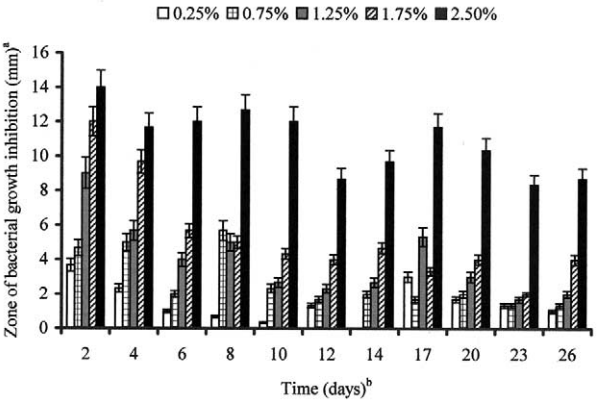

Fig 1. Effect of BAC (aqueous)-composite on *S mutans* growth. Measured zone of bacterial growth inhibition around BAC (aqueous)-composite samples on agar plates (*a*). Period in which BAC (aqueous)-samples were stored in sterile distilled water at 37°C (*b*). Composite samples were processed with diluted aqueous form of BAC.

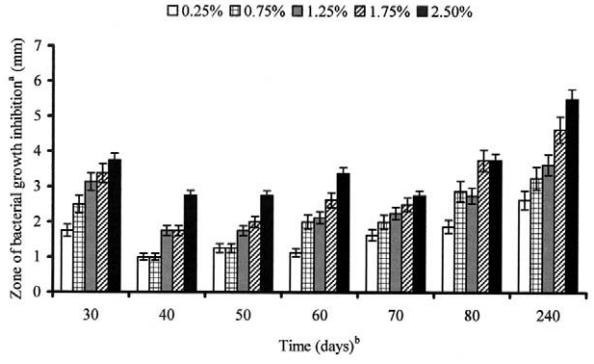

Fig 2. Effect of BAC (pure)-composite on *S mutans* growth. Measured zone of bacterial growth inhibition around composite samples on agar plates (*a*). Period in which samples were stored in water at 37°C (*b*). Composite samples were processed with pure form of BAC.

mutans, indicating no measurable antibacterial activity. However, the BAC (pure)-composite samples showed significant antibacterial activity when compared with the control ($P < .01$). Measurable zones of bacterial inhibition increased as the BAC content of test samples increased; the 0.25% sample demonstrated the least antimicrobial activity (Fig 2), and the 2.50% group showed the greatest.

When stored in distilled water, the BAC (pure)-composite retained antimicrobial activity for up to 240 days (Fig 2). All BAC (pure)-composites containing 0.25% to 2.50% of BAC demonstrated growth inhibition activity against *S mutans;* zones of bacterial

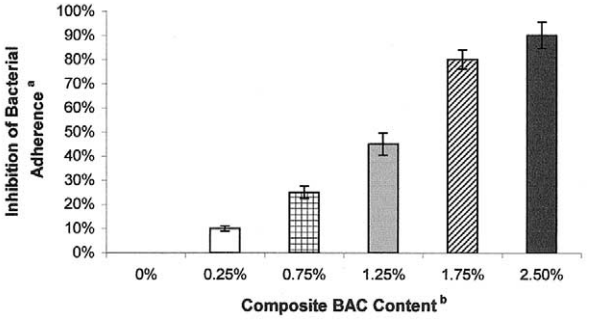

Fig 3. Effect of BAC-modified composite on inhibition of bacterial adherence. Percentage of inhibition = [1 - OD adherent S. mutans to BAC (pure)-composite] × 100%/OD adherent *S mutans* to control (*a*); weight percentage of diluted BAC in composite samples (*b*).

inhibition ranged from 1.75 mm (0.25%) to 3.75 mm (2.50%) at the end of 30-day storage. At day 80, the antimicrobial activity was comparable with that at day 30. At the end of 240 days of storage in distilled water, the BAC (pure)-composites retained their antimicrobial activity as demonstrated by the release of BAC into the surrounding agar surfaces. Composite materials containing high amounts of BAC (ie, 2.5%) showed higher antimicrobial activity than those with low (0.25%) BAC content. Samples that contained 0.25%, 0.75%, 1.25%, 1.75%, and 2.50% BAC had zones of bacterial inhibition measuring 1.75, 2.50, 3.12, 3.37, and 3.75 mm, respectively, at day 30; they maintained their antimicrobial activity at day 240 (Fig 2).

Bacterial adherence to BAC (pure)-composite samples

The effect of BAC on bacterial adherence to the composite sample was quantified by measuring the optical density of the adherent bacteria; increased optical density indicated an elevated bacterial count. The adherent bacteria were collected by dissolution in sodium hydroxide, and optical density was measured (550 nm). As shown in Figure 3, as BAC concentration increased, the adherence of *S mutans* decreased (10% to 90% inhibition). At a BAC concentration of 1.25%, up to 50% inhibition was observed; a BAC concentration of 2.50% resulted in more than 90% inhibition of the adherence of *S mutans* to BAC (pure)-composite.

Tensile bond strength of BAC (pure)-composite

Composite material must produce an adequate bond to be clinically acceptable. The tensile bond strength of the modified composite ranged from 9.38 to 9.75 MPa (Fig 4); 85% of debonding occurred at the composite

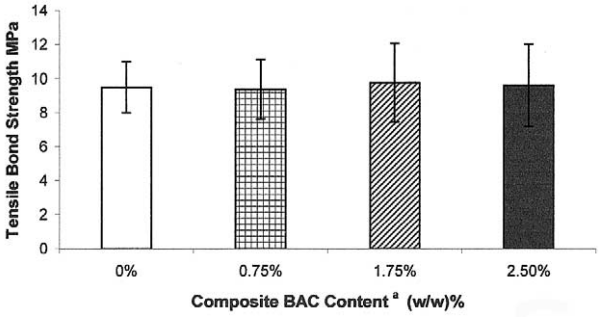

Fig 4. Tensile bond strength of BAC-modified composite samples. Weight percentage of BAC (pure) (*a*) that was mixed with bonding composite material (*b*).

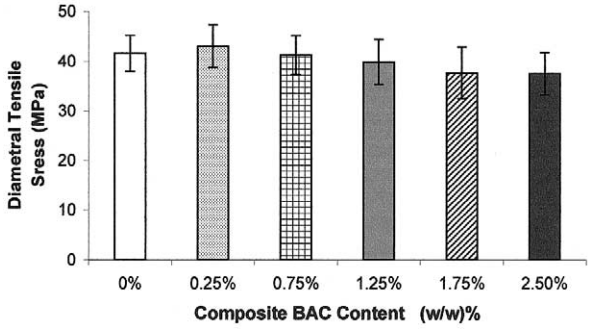

Fig 5. Diametral tensile strength of BAC (pure)-modified composite samples.

attachment interphase. One-way ANOVA showed no significant differences between the modified composite groups and the original product ($P < .05$), indicating that the addition of BAC does not affect the bonding properties of the composite.

Diametral tensile strength of BAC-modified composite

The diametral tensile strength test was used to measure the effect of BAC on the internal properties of the modified composite. The peak load to initiate failure (cracking or initial fracture) was determined with the universal testing machine; results for each sample were recorded in kilograms.

The diametral tensile strength for the samples ranged from 37.71 to 42.98 MPa (Fig 5). No significant difference was found among the control group and the modified composite groups ($P < .05$). A significant difference was found among the group containing 0.25% BAC and those containing 1.75% and 2.50% BAC.

DISCUSSION

Two types of adhesives are used in orthodontic bonding: resin composites and glass ionomer-based adhesives. Although composites are practically defenseless against microbial attack, glass ionomer cements have the advantage of fluoride release.[19] However, the leaking F^- ions weaken the matrix, and most studies conclude that the duration of fluoride release is very short. The antimicrobial properties of fluoride are limited, and its release occurs mainly beneath the orthodontic brackets, where often it is inefficient or unsuccessful in preventing decalcification. Many studies have reported that conventional glass ionomer cement is unacceptable clinically. This has led manufacturers to develop new resin materials that produce good bonds and release fluoride. Although some new products demonstrate superior bonding properties compared with conventional glass ionomer cement, their ability to release fluoride is limited and decreases significantly during the first month. In a prospective clinical trial,[20] a fluoride-releasing sealant was placed adjacent to previously bonded brackets in an attempt to lengthen fluoride release time. No significant differences in decalcification rates were found between the treatment and the control groups, and no added benefit with respect to reduced plaque or gingival irritation was demonstrated.

It has been previously shown that adding BAC to an orthodontic adhesive significantly increases its resistance to microbial attack.[11] However, the persistence of this effect and the antimicrobial properties of the adhesive have not been examined until now. The present study confirms that adding BAC to a composite material gives it antimicrobial properties. BAC release, as evidenced by antimicrobial activity, was found to be continuous and fairly constant over time (Fig 2), making it potentially desirable for clinical use. The continuous release of an antimicrobial agent would provide greater and more consistent protection against plaque and would make the modified composite more desirable than glass ionomer modified resins, which demonstrated limited effectiveness against white spot lesion formation.[1,13]

The original composite material used in this study contained fluoride, but no measurable antimicrobial property was noted for the control group. This suggests, although fluoride can facilitate remineralization of the enamel, that it possesses limited antimicrobial activity against the plaque that accumulates around bonded orthodontic appliances. The modified composite samples released BAC into the surrounding media throughout the study period (240 days), as indicated by the

zones of bacterial inhibition around the samples. After 8 months of storage in distilled water, the BAC-modified composites retained antimicrobial properties at levels comparable with those observed in the initial testing period. This consistency over time is important for composite materials used in long-term orthodontic treatment and might make the modified BAC composite more desirable than glass ionomers or resins containing glass ionomer, which demonstrated short-term release.

As described earlier, the limited benefits of adding fluoride to dental composites or glass ionomer materials led investigators to develop new materials that possess superior and sustained antimicrobial activity. Chlorhexidine has been incorporated into composite materials and glass ionomers, but it caused rapid deterioration of the bonding materials.

For strength, glass ionomer cement proved to be inferior to composite resin material.[14] Although the new hybrid glass ionomer cements are stronger than the old ones, they do not reduce decalcification significantly when compared with composite.

It is believed that adding antimicrobial agents does not alter the physical properties of the original composite material. In the present study, the physical properties of the BAC-modified composite remained comparable with the control (Figs 4 and 5). There was no significant difference between the tensile bond strength or the diametral tensile strength of the modified compounds and the control. The modified compounds demonstrated antimicrobial action and maintained the physical properties of the original composite material, making it favorable for clinical application.

This study did not investigate the effect of aging on the composite's physical properties. It has been reported previously that aging composite material stored in distilled water or saline solution shows a reduction in the modulus of rupture.[21] Future studies are needed to examine the effect of aging on BAC-modified composite.

Bonding the teeth of 1 patient (all maxillary and mandibular teeth mesial to first molars) would require approximately 180 mg of bonding composite; 0.50 to 4.6 mg of BAC would be needed to achieve 0.25% to 2.5% BAC concentration. BAC has been used as a preservative in ophthalmic solutions and contact lenses, but at concentrations lower than those used in our experiments; further studies on the cytoxicity and safety of the BAC-modified composite are needed before testing it clinically.

Based on the in vitro data obtained from this study, BAC-modified composite has the potential to be used clinically. Further in vivo evaluations of efficacy are essential. Various in vivo models are available to test fluoride's effect on enamel demineralization and remineralization. However, these models do not apply to BAC (pure)-composites, because the BAC does not affect enamel demineralization and remineralization. If the BAC (pure)-composites prove to be safe for oral use, their effect on in vivo plaque accumulation can be quantitatively determined with a plaque index. The in vivo method used by Ögaard et al[22,23] might also be used to test whether the BAC-modified composite can prevent the formation of white spot lesions.

CONCLUSIONS

The incorporation of BAC into a composite adhesive material added antimicrobial properties to the compound without affecting its mechanical properties. Additional studies are needed to test the clinical performance, safety, and efficacy of the modified composite and to find possible future clinical applications.

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