Original Article

Antibacterial Activity and Shear Bond Strength of 4-Methacryloxyethyl Trimellitate Anhydride/Methyl Methacrylate-Tri-*n*-butyl Borane Resin Containing an Antibacterial Agent

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ABSTRACT

Objective: To produce an antibacterial adhesive for orthodontic bonding without compromising the mechanical property.

Materials and Methods: We added benzalkonium chloride (BAC) to the Superbond C&B (4-methacryloxyethyl trimellitate anhydride/methyl methacrylate-tri-*n*-butyl borane [4-META/MMA-TBB]), a resin that exhibits a strong bonding strength between enamel and bracket. BAC concentrations in the BAC composites were 0.25%, 0.75%, 1.25%, 1.75%, 2.5%, and 5% (wt/wt). Antibacterial activity of the BAC composite was measured by the disk diffusion method. BAC-composite discs were placed on the surface of the agar inoculated with *Streptococcus mutans* and *Streptococcus sobrinus*, and the plates were incubated at 37°C. After 48 hours of incubation, the inhibition zone around each sample was measured and recorded. The BAC-modified composite was used to bond metal brackets to the phosphoric acid-etched enamel surface of human premolars. The shear bond strengths were measured after immersion in water at 37°C for 24 hours.

Results: The BAC-composite samples showed significant ($P < .0001$) antibacterial activity compared with the control. Measurable zones of bacterial inhibition increased as the BAC content in test samples increased. The shear bond strength declined with the increase in BAC concentration in the composite. A significant difference was found between the control composite and composites containing 1.25%, 1.75%, 2.5%, and 5% BAC ($P < .05$). No significant difference was found between the control composite and composites containing 0.25% and 0.75% BAC. However, shear bond strengths of the modified composites ranged from 10.12 MPa to 20.94 MPa.

Conclusions: These results confirmed that BAC-modified 4-META/MMA-TBB resin has a possibility for clinical application as an orthodontic bonding adhesive.

KEY WORDS: 4-META/MMA-TBB resin; Benzalkonium chloride; Antibacterial activity; Shear bond strength

INTRODUCTION

Currently, adhesive resin cements are widely used for bonding orthodontic brackets to enamel. Superbond C&B, a 4-methacryloxyethyl trimellitate anhydride/methyl methacrylate-tri-*n*-butyl borane (4-META/MMA-TBB) resin cement, is a unique MMA-based adhesive resin used widely for bonding orthodontic brackets and has earned a reputation for strong bonding.[1–4] It is reported that the variation of the concentration of phosphoric acid from 20% (wt) to 65% (wt) did not produce different bond strengths of 4-META/MMA-TBB resin to etched enamel, although demineralization decreased with increasing concentration of phosphoric acid. Thus, manufacturers recommend to preetch the enamel surface with 65% (wt) phosphoric acid for tight adhesion of the 4-META/MMA-TBB resin to the enamel in order to minimize the enamel loss.[5]

However, little attention has been paid to the antibacterial and biologic properties of adhesive resin cements such as 4-META/MMA-TBB resin. Good practice of oral hygiene for teeth fitted with bonded orthodontic brackets is important during orthodontic treatment. Orthodontic patients have been reported to have

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a higher incidence of white-spot lesions on the enamel around the bonded bracket than do control populations.[6–8] Some enamel damage at debonding has also been reported when an adhesive resin cement has been used.[9,10] The prevalence of at least one white-spot lesion found after bracket removal in patients with fixed appliances was 49.6% compared with 24% in untreated subjects.[11] Øgaard et al[12] indicated that a high prevalence of carries may be caused by the high cariogenic challenge prevailing in the plaque around orthodontic appliances. Proper oral hygiene is more difficult to maintain, and pH levels lower than 4.5 have been demonstrated in the plaques around the brackets and the bands during orthodontic treatment.[13]

Matasa[14] reported that orthodontic composite adhesives can host and nurture a variety of microorganisms and that their accumulation may lead to weakening of the bond and attack of the tooth. Currently, an orthodontic adhesive containing an antibacterial agent is not commercially available. To obtain antibacterial activity in glass-ionomer cements, several authors have attempted to incorporate chlorhexidine, and strong antibacterial activity caused by the release of the antibacterial agent was demonstrated.[15,16]

Yamauchi et al[17] reported that cured 4-META/MMA-TBB resin showed slight antibacterial effect. Kudou et al[18] have attempted to incorporate vancomycin and metronidazol in 4-META/MMA-TBB resin to obtain an antibacterial effect. Resin disks containing vancomycin showed a higher antibacterial effect against *Streptococcus mutans* than those containing metronidazol, without loss of bond strength to dentin. Kudou et al[18] provided no data for enamel. Recently, Kazuno et al[19] reported that MMA/TBB resin containing newly developed amphiphilic lipids showed a strong antibacterial effect against *S mutans* or *S sobrinus*.

Benzalkonium chloride (BAC) hand sanitizer is the most popular rinse-free hand sanitizer formula for normal hand washing.[20,21] Intranasal products containing the preservative BAC appear to be safe and well tolerated for both long-term and short-term clinical use.[22] Recently, Othman et al[23] confirmed that adding BAC to a composite material confers antibacterial properties. BAC release, as evidenced by antibacterial activity, has been found to be continuous and fairly constant over time, making it potentially useful for clinical application.

The purpose of the present study was to assess the antibacterial efficacy of 4-META/MMA-TBB containing BAC and finding the dose that does not compromise proper bond strength of the bracket to enamel.

MATERIALS AND METHODS

Superbond C&B resin cement (Sunmedical Co Ltd, Shiga, Japan) was used in this study. It was modified with addition of the antibacterial agent BAC (ICN Biomedicals Inc, Aurora, Ohio) in powder form.

BAC was initially diluted to 50% by mixing with Superbond C&B polymer. The polymer was added to the BAC-modified Superbond C&B that was later mixed with the monomer and catalyst according to the manufacturer's instructions. The final BAC concentrations in the BAC composites were 0.25%, 0.75%, 1.25%, 1.75%, 2.5%, and 5% (wt/wt). Disks of uniform size (8.0 mm diameter $\times$ 2.0 mm thick) were made from all the BAC composites with custom-made molds. Oral cariogenic streptococci, *S mutans* 10449 and PS14 as well as *S sobrinus* 6715 and B13, were used as the test bacteria. They were grown routinely in a brain-heart infusion medium (Difco, Detroit, Mich) at 37°C overnight.

Antibacterial Activity of BAC Samples

A disk diffusion assay was used for the evaluation of antibacterial activity. The release of BAC into the surrounding agar medium as shown by growth inhibition of *S mutans* and *S sobrinus* was evaluated. The growth effect was determined by measuring the semi-diameter of the zone of growth inhibition around the BAC-composite disc.

An overnight broth culture of *S mutans* or *S sobrinus* was diluted and the cell suspension was adjusted to an optical density of 0.5 (550 nm). Then brain-heart infusion agar plates were inoculated with 80 μL of a 60-fold dilution of the cell suspension. The inoculum was spread evenly on the plate surface with a glass rod to provide uniform bacterial growth. BAC-composite discs were placed on the surface of the agar, and the plates were incubated at 37°C. After 48 hours of incubation, the inhibition zone around each disc sample was measured and recorded.

Bonding Procedures

A total of 70 extracted human premolars were used in this study. They were randomly placed into seven groups and were embedded in acrylic resin with the buccal surfaces available for bonding. After acrylic-resin curing, the tooth surfaces to be bonded were cleansed and then polished with pumice and rubber prophylactic cups for 10 seconds in order to simulate routine clinical procedures.

Orthodontic metal brackets (Super mesh STD Edgewise 131–45B, Tomy International Inc, Tokyo, Japan) were used in this study. The average bracket surface area was determined to be 11.188 mm^2. A tooth was etched with 65% phosphoric acid gel (which is included in Superbond C&B kit) for 30 seconds, washed for 20 seconds, and air dried. Then, a metal orthodontic bracket was bonded to the etched enamel surface with

Table 1. Disk diffusion assay[a]

BAC concentration (wt%)	*Streptococcus mutans*				*Streptococcus sobrinus*			
	PS14		10449		6715		B13	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control (0%)	0.39 A	0.79	0.78 F	0.98	0.16 L	0.32	1.16 R	2.32
0.25	5.36 B	0.91	6.32 G	0.79	6.00 M	0.59	5.34 S	1.15
0.75	6.26 BC	1.48	5.83 GH	2.12	6.00 MN	1.53	6.41 ST	1.14
1.25	6.65 BCD	0.88	7.97 GHI	1.03	6.91 MNO	0.65	7.18 STU	1.22
1.75	8.10 CD	1.84	9.01 IJ	1.16	9.18 OP	1.20	9.12 TUV	1.81
2.50	12.00 E	2.82	10.74 J	1.17	11.16 P	2.98	10.89 V	3.22
5.00	14.29 E	1.97	16.54 K	2.32	14.58 Q	3.13	14.10 W	2.30

[a] BAC indicates benzalkonium chloride; SD, standard deviation. Data are measured semidiameter of bacterial (*S mutans* and *S sobrinus*) growth inhibition around the BAC-composite discs on agar plates (mm). No significant differences existed among the bacterial strains within the same BAC concentration ($P > .05$). Significant differences existed among the different BAC concentrations in the composite ($P < .05$). Mean values with different letters at the same bacterial strain are significantly different ($P < .05$).

Table 2. Shear bond strengths (MPa)[a]

Composite BAC content (wt %)	Mean	SD	Range
Control (0%)	21.78 A	4.06	16.20–27.85
0.25	20.94 AB	1.71	17.41–22.78
0.75	18.52 AB	3.47	10.86–22.78
1.25	18.29 BC	4.43	10.35–22.78
1.75	15.18 CD	3.41	10.51–20.53
2.50	13.84 D	3.82	8.38–18.78
5.00	10.12 E	3.08	6.14–15.48

[a] BAC indicates benzalkonium chloride; SD, standard deviation. Mean values with different letters are significantly different ($P < .05$).

BAC-composite resin cement (containing 0%, 0.25%, 0.75%, 1.25%, 1.75%, 2.5%, or 5% BAC). The catalyst, a partly oxidized TBB initiator, was added to the monomer mixture of 4-META and MMA to prepare an activated polymerized monomer liquid. Then, the polymer powder and activated monomer liquid were mixed and used to bond metal brackets to the treated enamel surface by the brush-dip technique.

Each bracket was subjected to a 300 g force, according to the report of Bishara et al,[24] and excess bonding resin was removed with a small scaler.

Shear bond strength was measured according to the methods recommended by the International Organization for Standardization[25] with a testing machine (TCM-500CR, Shinkoh, Tokyo, Japan) at a crosshead speed of 2 mm/min.

After debonding, the teeth and brackets were examined under 10× magnifications. The debonding condition of each specimen was scored by using the adhesive remnant index (ARI).[26] The ARI scores ranged from 0 to 3 as follows: score 0 = no adhesive remained on the enamel, 1 = less than half of the adhesive remained on the tooth surface, 2 = more than half of the adhesive remained on the tooth, and 3 = all the adhesive remained on the tooth with a distinct impression of the bracket base. Enamel fracture was also scored according to the method of Schaneveldt and Foley.[27]

Statistical Analysis

The data were presented in mean and standard deviation. One-way analysis of variance (ANOVA) was used to determine whether a significant difference existed among various groups, and Fisher test was used for multiple comparisons. Chi-square test was used to detect statistical differences in ARI scores among the seven protocols. Significance for all statistical tests was predetermined at $P < .05$.

RESULTS

Disk Diffusion Assay Method

The results of disk diffusion assay method are listed in Table 1. Two-way ANOVA showed significant differences among different BAC concentrations in the composites ($F = 104.773$, $P < .0001$). However, no significant differences existed among the bacterial strains ($F = 0.591$, $P = .6225$). Two-way interactions were not found for the BAC concentration and bacterial strain ($F = 0.476$, $P = .9618$).

The composite without BAC used as the control demonstrated almost no inhibition zone when placed on an agar plate preinoculated with *S mutans* or *S sobrinus*. However, the BAC-composite discs showed significant antibacterial activity when compared with the control ($P < .0001$). Their antibacterial activity increased as the concentration of BAC increased. Measurable zones of bacterial inhibition increased as the BAC content in the test samples increased. Samples containing 5% BAC exhibited the highest antibacterial activity.

Comparison of Shear Bond Strengths

The results of shear bond strength measurements (MPa) are listed in Table 2. One-way ANOVA showed significant difference in bond strength among different BAC concentrations in composites ($F = 12.448$, $P < .0001$). However, shear bond strength of the modified composite ranged from 10.12 MPa to 20.94 MPa.

The shear bond strength declined as the BAC concentration increased. A significant difference was found between the control composite and the composites containing 1.25%, 1.75%, 2.5%, or 5% BAC ($P < .05$). No significant difference was found between the control group and the composites containing 0.25% and 0.75% BAC.

Comparison of ARI

All ARI scores after debonding are ARI = 0 for different BAC-modified 4-META/MMA-TBB resin including the control. The resin was observed only on the bracket after debonding for all BAC concentrations. Chi-square test showed no significant difference in ARI scores among the seven materials.

DISCUSSION

More than 500 bacterial groups are present in the oral cavity, but relatively few species are related to caries or pulpal infections.[28] Among these streptococci, *S mutans* and *S sobrinus* are the principal species associated with caries in humans.

S mutans strains are predominant and often found alone, whereas *S sobrinus* strains are usually detected in individuals also harboring *S mutans*. However, recent epidemiologic studies have indicated that *S sobrinus* is more frequently isolated from highly caries-susceptible patients compared with *S mutans* and that the cariogenic potential of *S sobrinus* is greater than that of *S mutans*.[29,30] We used BAC as an antibacterial agent that is active against both bacteria.[23]

The present study confirms that the addition of BAC to 4-META/MMA-TBB resin confers antibacterial properties, and the resulting composite has strong antibacterial activity because of the release of the antibacterial agent against *S mutans* and *S sobrinus*.

The clinical acceptable shear bond strength remains unknown. Saito et al[31] have shown that the bonding durability of a 4-META/MMA-TBB resin does not weaken to less than 10 MPa when phosphoric acid etching is used. In this study, the shear bond strength of the modified composite ranged from 10.12 MPa to 20.94 MPa. These results suggest that BAC-modified 4-META/MMA-TBB resin has a possibility for clinical application as an orthodontic bonding adhesive, although further studies are required to establish the clinically acceptable shear bond strength. In the clinical situation, long-term release of the antibacterial agent is essential. The release behavior of BAC should be further investigated.

A bonding procedure in one patient requires approximately 150 mg of bonding composite, and 0.7 mg of BAC is needed to achieve 0.5% BAC concentration. BAC has been used as an antiseptic in contact lenses, but the quantity used in the present study was less than that used in contact lenses. It is necessary to conduct further research on the cytotoxicity and safety of the BAC-incorporated 4-META/MMA-TBB resin before it can be tested clinically.

CONCLUSIONS

a. Cured 4-META/MMA-TBB resin has little antibacterial effect.
b. The addition of BAC to 4-META/MMA-TBB resin confers an antibacterial effect while retaining sufficient bond strength.

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