

Benzalkonium chloride in an orthodontic adhesive: Its effect on rat enamel demineralization using color-based image analysis

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Introduction: The aim of this study was to determine the effect of an orthodontic bonding adhesive containing benzalkonium chloride (BAC) on enamel demineralization. Methods: Eighteen female Sprague-Dawley rats, aged 8 to 10 weeks, were inoculated with *Streptococcus sobrinus* for 5 days. The animals were randomly divided into the control, non-BAC, and BAC groups. The 6 animals in each group did not receive any brackets, received brackets on the maxillary left first molars bonded with conventional adhesive, or received brackets on the maxillary left and right first molars bonded with adhesive incorporated with 0.25% and 0.75% BAC (wt/wt), respectively. After 7 weeks, the maxillae were soaked in murexide stain to observe the surface area (mm²) and percentages of enamel demineralization on the palatal, mesial, buccal, and occlusal surfaces of the maxillary molars using color-based image analysis. **Results:** The non-BAC and BAC groups exhibited greater enamel demineralization compared with the control group. The surface areas and percentages of enamel demineralization in the BAC group were less compared with the non-BAC group. Less enamel demineralization was noted in the animals treated with 0.75% BAC compared with those given 0.25% BAC in all areas; however, these differences were not great enough to attain statistical significance at the 0.05 level. **Conclusions:** The addition of BAC to an orthodontic composite has the potential to reduce the amount and percentage of enamel demineralization. In addition to being an antibacterial agent, BAC may also have an anticariogenic effect. Increased sample sizes and testing of more concentrations of BAC are recommended. (Am J Orthod Dentofacial Orthop 2019;155:88-97)

Enamel demineralization occurs more often among orthodontically treated patients than in those who never had orthodontic treatment.¹ White spot lesions from enamel demineralization can develop around

brackets as early as 4 weeks after the initiation of orthodontic treatment,² and prevalence rates of 72.9%³ and 75.6%⁴ among orthodontic patients have been reported. A reason for the high incidence of enamel demineralization may be the presence of metallic orthodontic brackets, which are associated with decreased pH levels of saliva, increased plaque accumulation,⁵ and elevated colonization of *Streptococcus mutans*.⁶ Oral hygiene maintenance for orthodontic patients has been found to be ineffective in preventing enamel demineralization, and there is a need to discover and further investigate other intervention and preventive strategies to reduce this common problem in patients undergoing orthodontic treatment.³

The antimicrobial effects of fluoride,^{1,7} chlorhexidine,⁸⁻¹⁰ and cetylpyridinium chloride,¹¹ when incorporated into orthodontic adhesives, have been studied and found to be advantageous.¹² Benzalkonium chloride (BAC) is currently 1 of the

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most beneficial antimicrobial agents being used. A quaternary ammonium compound, BAC causes structural disorganization, loss of cytoplasmic membrane integrity, and other destructive effects to bacteria.¹³ The applications of BAC are wide ranging, since it is used in disinfectants, ophthalmic drops, skin antiseptics, hand sanitizers, wet wipes, diaper creams, and food preservatives.¹⁴

In orthodontics, in-vitro studies evaluating the antibacterial effect,¹⁵⁻¹⁷ cytotoxic effect,¹⁷ and effect on mechanical properties of BAC, when incorporated in orthodontic resin,¹⁶ have been performed. BAC increased the antimicrobial properties of an orthodontic adhesive without a statistically significant decrease in bond strength after 24 hours or 25 days. The maximum release and minimum inhibitory concentration levels of BAC were found to be higher when compared with chlorhexidine or triclosan.¹⁸ However, as the BAC concentration was raised, a decline in shear bond strength was observed.¹⁶ Nevertheless, the documented shear bond strengths of 10.12 to 20.94 MPa when BAC was incorporated in the orthodontic adhesive were still higher than the normal forces of mastication that are approximately 5.9 to 7.8 MPa.¹⁹ Another in-vitro study confirmed that BAC in an orthodontic adhesive has an antibacterial effect against *Streptococcus mutans* and *Streptococcus sobrinus*. Concentrations of 0.25% and 0.75% exhibited cytotoxicity similar to the resins without BAC or the controls when exposed to human gingival cells.¹⁷

It is presumed that the oral environment will influence the release of BAC and its antibacterial activity.¹⁵ Previous studies did not directly correlate BAC's antibacterial activity to a reduction of demineralization or to an anticariogenic potential. Since the carious process is a dynamic alteration of demineralization and remineralization, evidence of a decrease in demineralization may indicate BAC's ability to prevent caries. Therefore, the aim of this study was to determine the effect of an orthodontic composite resin containing BAC on enamel demineralization around orthodontic brackets in an animal model.

MATERIAL AND METHODS

A protocol of the experiment, including those pertaining to animal-related procedures, was reviewed and approved by the Institutional Animal Care and Use Committee of the National Institutes of Health of the University of the Philippines, Manila (protocol number, 2015-018) and by the Republic of the Philippines Department of Science and Technology (reference number, AR-2016-189).

A sample of 8- to 10-week-old female Sprague-Dawley albino rats, with weights of 180 to 220 g, was

acquired from the Republic of the Philippines Food and Drug Administration and underwent the Body Condition Scoring Method before allocation in solid-floor plastic transport cages with their social group.²⁰ The animals had a body condition of 3, a well-conditioned body, before being transferred to the Laboratory Animal House Facility of the Department of Science and Technology.

The animals were randomly distributed into 3 groups of 6 rats each: control group, non-BAC group, and BAC group. They were acclimatized for 7 days and given a rodent diet (Bio-300; Purina, Cargill Philippines, Bulacan, Philippines) and distilled nonfluoridated water. They received a daily dose of multivitamins and were disturbed as little as possible, except when feeding and changing the water. Acclimatization was confirmed by the body condition scoring technique.²⁰

S sobrinus Coykendall (NIDR 6715-7), from the American Type Culture Collection (Manassas, Va), was transported as lyophilized cultures in a freeze-dried microbial preparation on dry ice in plastic cryopreservation vials. All containment requirements appropriate for the biosafety of the cultures, such as the Class II vertical laminar flow microbiologic safety cabinets (Safe FAST Elite; Faster, Cornaredo, Italy), were used for personnel and environmental protection. Brain-heart infusion broth was used as the medium for bacterial propagation. Confirmation of bacterial concentration was done using a spectrophotometer (CT-2700 Biobase; E Chrom Tech, Taipei, Taiwan) to measure the wavelength, absorbance, and transmittance of the suspensions. Before inoculation, the bacterial suspension had the infectious dose with a concentration of approximately 4.5×10^8 CFU per millileter of *S sobrinus*. To achieve the desired concentration, bacterial propagation began 18 hours before each day of inoculation. The suspension was used within 4 hours to prevent any major change in bacterial concentration. To ensure even distribution of the bacteria in the suspension, a vortex mixer was used before the inoculation of each animal.

Each rat was weighed to compute the necessary anesthetic dosage of 0.1 mL per kilogram of body weight. To induce anesthesia, 125 mg of Tiletamine hydrochloric acid and 125 mg Zolazepam (Zoletil 50; Virbac, Carros, France) were intramuscularly administered. Approximately 0.2 to 0.5 mL of the bacterial suspension was swabbed around the oral cavity, particularly on the tongue, teeth, and cheeks of all animals, with a microbrush. During bacterial inoculation, the animals were fed with rodent diet and given 1 mL of the bacterial suspension in the drinking water.

Animals in the control group served as the infected control and received no brackets. The non-BAC group

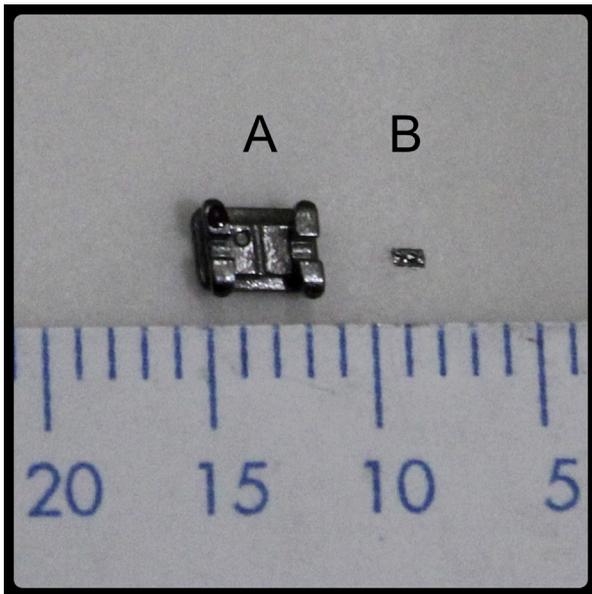


Fig 1. A, Regular orthodontic bracket compared with B, a miniature bracket used in the study.

received miniature orthodontic brackets bonded with a conventional (without BAC) orthodontic adhesive on the mesiopalatal area of the maxillary left first molar. The BAC group received miniature orthodontic brackets bonded with 0.25% BAC-infused adhesive on the mesiopalatal area of the maxillary left first molar and 0.75% BAC-infused adhesive on the mesiopalatal area of the maxillary right first molar.

Benzalkonium chloride (Kao Industrial, Chonburi, Thailand) was purchased from a local distributor. Measurement and incorporation of BAC was done at the College of Chemistry, University of the Philippines, Diliman, producing 0.25% BAC on the low-dose side and 0.75% BAC on the high-dose side. This was done 1 day before bracket placement to ensure that no premature setting or hardening of the activator occurred.

An improvised mini-cheek retractor made of bent 0.030-in stainless steel wire was used to isolate the molars. Atropine sulfate (anespin) was administered to control salivation during bracket placement. The animals were given a cariogenic diet of 67% sucrose and distilled nonfluoridated water with 10% sucrose ad libitum. This diet was based on the MIT-200 diet used by Navia²¹ and was reproduced by combining locally purchased rodent pellets (Bio-300) and refined table sugar.

Miniature orthodontic brackets were cut from prefabricated stainless steel lingual retainers (US Dotics, Manila, Philippines) using an orthodontic wire cutter. All dimensions of the brackets were equal to or



Fig 2. A miniature orthodontic bracket (arrow) bonded on the mesiopalatal area of the maxillary left molar.

less than 1.0 mm (Fig 1). The thickness of the brackets was estimated to be equal to or less than 0.5 mm.

Self-curing orthodontic adhesive (System 1 Plus; Ormco, Glendora, Calif) was used in the study. For the non-BAC and BAC groups, the designated tooth surfaces were conditioned by cleaning and etching using 37% orthophosphoric acid (System 1 Plus) for 30 seconds before bonding. The adhesive activator was applied on the tooth surfaces using a microbrush. The corresponding adhesive was placed on the mesh side of the brackets with a dental explorer. For the non-BAC group, conventional adhesive was used to bond the brackets on the mesiopalatal area of the maxillary left first molar (Fig 2). For the BAC group, 0.25% BAC-infused adhesive was used to bond the brackets on the mesiopalatal area of the maxillary left first molar, and 0.75% BAC-infused adhesive was used to bond the brackets on the mesiopalatal area of the maxillary right first molar.

Once a week, the animals were orally inoculated with *S sobrinus* and inspected for debonded brackets. Debonded brackets were replaced with new ones and bonded with the corresponding adhesive. All bonded brackets were debonded and replaced every week.

After 7 weeks, the animals were killed with carbon dioxide. This was done at the necropsy room of the animal house of the University of the Philippines. After death was confirmed, the animals were decapitated to detach the maxillae, remove all soft tissues, and air dry the surfaces (Fig 3).



Fig 3. Left part of the maxilla after removal of soft tissue before murexide staining.

The maxillae were immersed in a staining solution of murexide (Sigma Aldrich, St Louis, Mo). The staining solution was mixed at the testing division of the College of Chemistry at the University of the Philippines, Diliman. To create the staining solution, 60 mg of murexide powder was incorporated into 75 mL of distilled water and 175 mL of absolute ethanol. The solution was quickly filtered in a vacuum. A 0.45- μ m nylon membrane filter (Whatman, Buckinghamshire, United Kingdom) was used to separate the supernatant that was used as the staining solution. The dried maxillae were immersed in this solution and stained for 16 hours.

A digital single-lens reflex camera (EOS 550D; Canon, Tokyo, Japan) with lens type EF-S 18 to 55 mm., at Aperture Priority mode, without flash, with exposure time of 1/80 second, and ISO speed of 500, was used to capture the image of the palatal, mesial, buccal, and occlusal surfaces of the maxillary molars. To ensure that the distance and angle between the camera lens and the lingual surfaces were the same for all subjects, the camera was attached to a secured steel contraption, and a remote control was used to take the photos. The maxillary bone, along with the teeth, were colored with a black permanent marker to isolate the molars in the photographs and then mounted on a labeled black square background over 1-mm gridlines using black modeling clay (Fig 4). All digital photos were analyzed at the Department of Computer Science at the University of the Philippines, Diliman, for color-based image analysis.

The photos underwent preprocessing using thresholding, connected components analysis, and a flood-fill algorithm to segment the tooth areas. Binary thresholding was used to segment the black square containing the tooth sample. Any pixel with a hue above 40 was considered as background, retaining everything else. Connected components analysis was performed to group neighboring nonblack pixels for segmentation from the background. The largest groups from the results of connected components analysis underwent

another thresholding to segment the outline of the tooth. Lastly, the flood-fill algorithm was applied to the segmented outline to produce a mask corresponding to the tooth areas.

Demineralized areas on the teeth were in the color range of dark pink to light pink. These areas were extracted from the white areas (sound enamel) to estimate the extent of demineralization. Since the identification is single color-based with varying intensities, the color space used was hue-saturation-value. The pixels whose hue $\in [0,11]$, saturation $\in [65,255]$, and value $\in [180,250]$ considered to be demineralized areas (Fig 5).

To estimate each sample's surface area with its 2-dimensional projection, a millimeter was estimated in pixels from the data set. Sample grids were extracted from the set of images and simplified to black and white; those with pixel values from 200 to 255 were converted to white, and those with pixel values from 0 to 199 were converted to black. The conversion of an image to black and white automatically removed noise and unwanted information from the digital image. This transformation converted the images to measurable binary form that could be further enhanced to create more solid lines. Binarization plays an important part in improving, recognizing, and measuring images.²²⁻²⁴

The estimated pixels per 1 mm² in the data set, given the parameters of the camera and the setting of the gathering site, were 2450 pixels. This value was used to calculate the overall estimated surface area of the teeth, the demineralized pink areas, and the white areas. The percentage of enamel demineralization was computed by dividing the surface area of enamel demineralization by the surface area of the entire maxillary molars and then multiplying the quotient by 100.

Statistical analysis

Missing data on the surface area of demineralized enamel was observed in 2 animals given 0.75% BAC. Data from 1 animal were missing at the buccal area and from another at the occlusal area. Missing values were imputed using regression methods with group and specific animal as regressors. This was performed using the IMPUTE module in Stata software (version 10.1; StataCorp, College Station, Tex). After imputing the missing values, the means and standard deviations of the actual and percentage surface areas of demineralized enamel were obtained.

Since both 0.25% and 0.75% were applied in the same rat, statistical significance of the mean differences between treatment groups (control, non-BAC, and BAC) were done using analysis of variance (ANOVA) for a nested design where the rat effects were nested in the

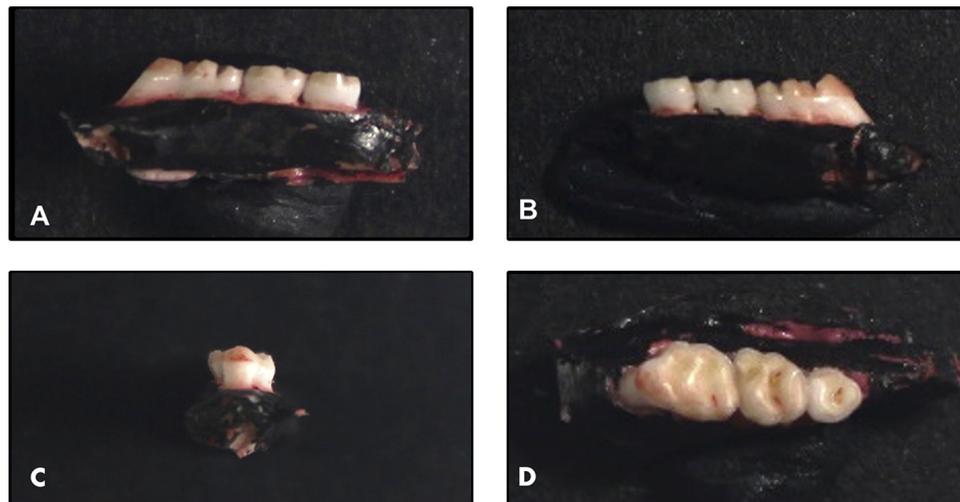


Fig 4. Tooth surfaces after murexide staining: **A**, palatal area; **B**, buccal area; **C**, mesial area; **D**, occlusal area.

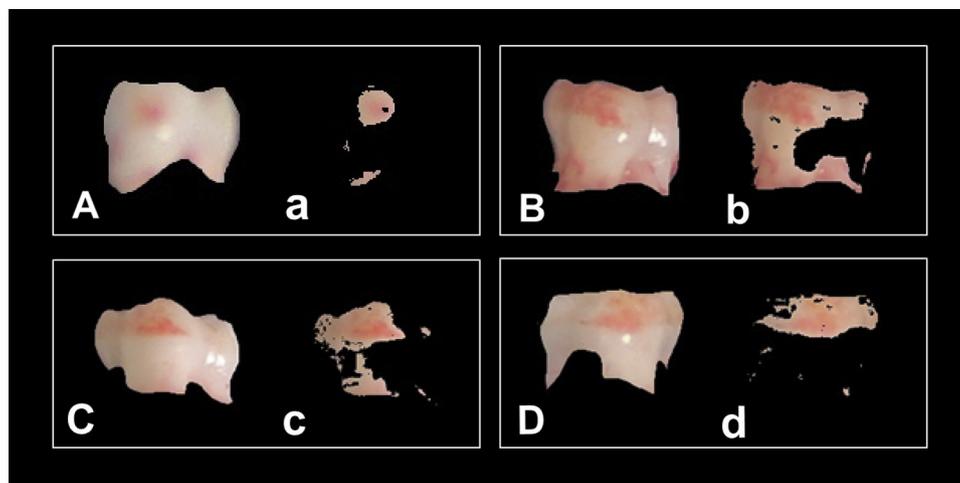


Fig 5. Mesial areas of the maxillary first molars from the groups before color-based image analysis extraction: **A**, control group; **B**, non-BAC group; **C**, 0.25% BAC group; **D**, 0.75% BAC group. Images of the same areas after color-based image analysis extraction: **a**, control group; **b**, non-BAC group; **c**, 0.25% BAC group; **d**, 0.75% BAC group.

treatment group effects. The variation due to the effect of individual animals was used as the error term in determining statistical significance of the variation contributed by treatment groups in ANOVA. To control for type I error in multiple comparisons testing, the Tukey honest significant difference was used to determine which pair of treatment groups differed significantly from the others. For this procedure, the mean of the surface areas of the 0.25% and 0.75% BAC treatments were first averaged in each animal given these treatments to represent the values for the BAC group.

Possible effects of heteroscedasticity (unequal variances) were accounted for in the pairwise comparisons by using the Welch standard error and the Satterthwaite approximate degrees of freedom. This was done by invoking the “unequal” option in the *prcomp* module in the Stata software.

Finally, the paired *t* test was used to test the differences in the areas of demineralized enamel between 0.25% BAC and 0.75% BAC. The level of significance for all analysis was $\alpha = 0.05$. Statistical analyses were generated using the Stata software.

Table I. Power analysis for sample size of 5 rats per group with 5 level of significance

Difference to be detected (%)	Standard deviation of percentage of enamel demineralization (%)	Power (%)
20	5	>99.9
15	5	99.7
10	5	88.5
5	5	35.3
20	10	88.5
15	10	66.0
10	10	35.3
5	10	12.4

Table I shows the results of power calculations for a sample size of 5 rats per group at the 5% level of significance. These calculations show that, if the standard deviation of the percentage of enamel demineralization is 10%, there is 88.5% power to detect a significant difference if the actual difference between 2 groups is 20%. There is low power to detect differences of 15% or less between 2 groups if the standard deviation is 10%. If the standard deviation is 5%, there is enough power to detect actual differences of 10% or higher.²⁵

RESULTS

Of the initial 18 animals tested, 2 from the non-BAC and BAC groups died 2 days before they were to be killed. The data generated from both animals were included in the analysis. Data on surface area (mm²) and percentage of demineralized enamel for each treatment group at different surfaces of the maxillary molars are shown in Figure 6. The variability of these measurements was considerably large with coefficients of variation exceeding 50% in most distributions (Table II).

The ANOVA results indicated significant differences in the means among the treatment groups for both surface area (mm²) and percentage of demineralized enamel. Overall, the animals that received orthodontic brackets (non-BAC and BAC groups) had greater surface areas and percentages of enamel demineralization on all surfaces observed, compared with the animals that did not receive brackets (control group). There was less enamel demineralization in the brackets with BAC compared with the brackets without BAC (Table II).

The testing for significant differences between all pairs of mean surface areas and percentage of demineralized enamel using the Tukey HSD test are summarized in Table III. Demineralized enamel in the non-BAC group was significantly higher than in the control group for all tooth surfaces at the 0.05 level of significance. There were small and not significant differences in the mean surface areas and percentages of demineralized enamel

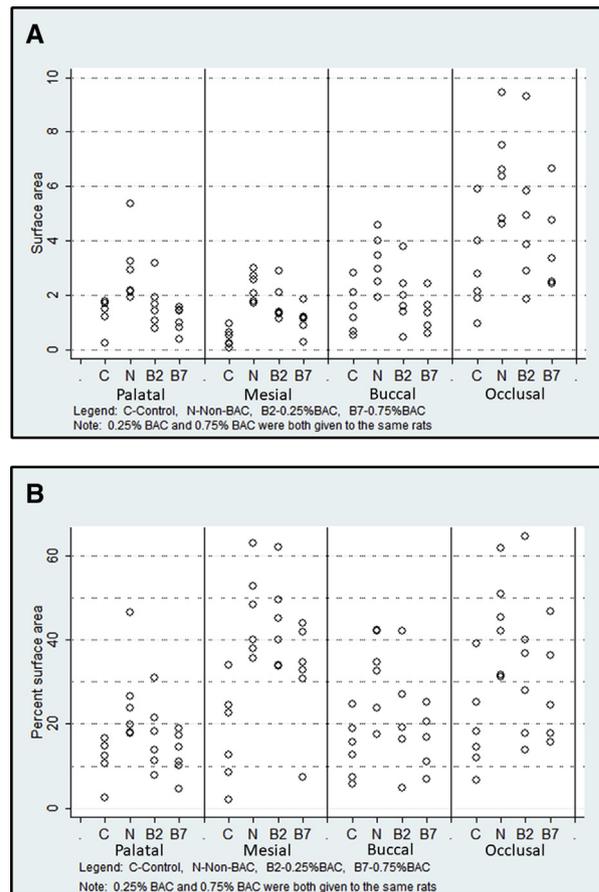


Fig 6. A, Surface area (mm²) of enamel demineralization on surfaces of rats' maxillary molars with different treatments; B, percentages of surface area of enamel demineralization of maxillary molars with different treatments.

between the control and BAC groups on the palatal, buccal, and occlusal areas. It was only on the mesial area where the size of demineralized enamel of the BAC group was statistically larger than the corresponding size of the control group, both in terms of surface area (d = 0.96 mm²) and percent demineralization (d = 20.6%). The surface area of enamel demineralization was significantly lower in the BAC group, compared with that in the non-BAC group for all surfaces, except for the occlusal surface. When percent demineralization was compared between the non-BAC and BAC groups, only the buccal surface demonstrated a significant difference (d = 13.43%).

The mean surface areas and percentages of demineralized enamel in the 0.25% and 0.75% BAC groups were assessed for significant differences using the paired t test (Table IV). Generally, there was less enamel demineralization in the animals treated with 0.75% BAC compared with those given 0.25% BAC in

Table II. Comparison of mean surface areas (mm²) and percentages of enamel demineralization of tooth surfaces in the 3 groups

Tooth surface	Group	n	Surface area				Percentage			
			Mean (mm ²)	SD (mm ²)	ANOVA F-test	P	Mean (%)	SD (%)	ANOVA F-test	P
Palatal	Control	6	1.12	0.70	7.01	0.0071	9.85	6.11	5.01	0.0220
	Non-BAC	6	2.96	1.28			25.40	10.94		
	BAC	12	1.38	0.72			15.02	7.03		
Mesial	Control	6	0.44	0.33	17.97	0.0001	17.39	11.75	9.38	0.0020
	Non-BAC	6	2.31	0.54			46.27	10.49		
	BAC	12	1.40	0.65			37.99	13.06		
Buccal	Control	6	1.48	0.87	8.26	0.0038	14.16	7.23	8.01	0.0040
	Non-BAC	6	3.25	0.98			32.20	9.99		
	BAC	12	1.67	0.92			18.76	9.83		
Occlusal	Control	6	2.94	1.77	4.28	0.0339	19.31	11.56	4.10	0.0380
	Non-BAC	6	6.57	1.80			43.86	11.71		
	BAC	12	4.44	2.12			31.39	14.96		

Table III. Pairwise comparisons of the surface areas (mm²) and percentages of demineralized enamel of the tooth surfaces

Tooth surface	Group	Surface area (mm ²)		Percentage	
		Mean difference (d)	Critical difference	Mean difference (d)	Critical difference
Palatal	Control vs non-BAC	-1.85*	1.38	-15.56*	11.83
	Control vs BAC	-0.27	0.82	-5.17	7.81
	Non-BAC vs BAC	1.58*	1.36	10.36	11.82
Mesial	Control vs non-BAC	-1.87*	0.59	-28.86*	14.35
	Control vs BAC	-0.96*	0.55	-20.6	13.93
	Non-BAC vs BAC	0.91*	0.66	8.27	13.01
Buccal	Control vs non-BAC	-1.77*	1.19	-18.04*	11.37
	Control vs BAC	-0.19	0.95	-4.61	8.18
	Non-BAC vs BAC	1.58*	1.05	13.43*	10.71
Occlusal	Control vs non-BAC	-3.63*	2.29	-24.54*	14.97
	Control vs BAC	-1.49	2.41	-12.08	16.58
	Non-BAC vs BAC	2.13	2.42	12.47	16.66

*Mean difference is statistically significant at the $\alpha = 0.05$ level.

all areas. These differences, however, were not large enough to attain statistical significance at the 0.05 level.

DISCUSSION

The formation of dental caries is a dynamic and multifactorial process involving several variables: cariogenic bacteria, substrate, tooth surface, and adequate time for the lesion to develop.^{26,27} Fixed orthodontic appliances have been proven to enhance the accumulation of plaque, increase the difficulty of maintaining good oral hygiene, and hamper salivary self-cleaning.²⁸⁻³⁰

In this study, *S sobrinus* was the organism used to infect the animals and produce enamel demineralization. Human dental plaque was found to have as much as 83% prevalence rate of *S sobrinus*,³¹ occurring more frequently on molars and smooth surfaces.³² Its presence was found to accelerate the development

of caries by 5 times, 1 year after it was detected, making it an important risk factor because of its aggravating effect.³³ *S sobrinus* and *S mutans* can be considered "equally virulent with regard to dental caries."³⁴

One limitation encountered in this study was the lack of available equipment to confirm the presence of *S sobrinus* infection. Hence, instead of inoculating the animals for 3 days only, a 5-day inoculation period, similar to a study by Dinis et al,³⁵ was used. In addition, the animals were inoculated once a week during inspection of the brackets. We used the bacterial concentration of 4.5×10^8 CFU per milliliter used in the study of Dubroc et al.³⁶

BAC, in concentrations of 0.25% and 0.75%, was used in the study, since these amounts had clinically acceptable ranges for shear bond strengths and cytotoxicity based on the studies of Saito et al.^{16,17}

Table IV. Mean surface areas (mm²) and mean percentages of demineralized enamel on the different areas with 0.25% BAC and 0.75% BAC

Tooth surface	Group	n	Surface area		Paired t test	P	Percentage		Paired t test	P
			Mean (mm ²)	SD (mm ²)			Mean (%)	SD (%)		
Palatal	0.25% BAC	6	1.68	0.85	1.789	0.135	17.27	8.29	1.594	0.172
	0.75% BAC	6	1.10	0.46			12.78	5.28		
Mesial	0.25% BAC	6	1.69	0.68	2.123	0.087	44.07	10.74	2.118	0.088
	0.75% BAC	6	1.09	0.51			31.93	13.11		
Buccal	0.25% BAC	6	1.94	1.13	0.864	0.427	20.99	12.54	0.634	0.554
	0.75% BAC	6	1.40	0.63			16.54	6.59		
Occlusal	0.25% BAC	6	4.78	2.62	0.867	0.426	33.48	18.36	0.761	0.481
	0.75% BAC	6	4.09	1.64			29.29	12.03		

In August 2016, the Food and Drug Administration of the Department of Health and Human Services in the United States issued a final rule on the Safety and Effectiveness of Consumer Antiseptics.³⁷ It banned 19 chemicals, including 5% to 10% povidone-iodine and triclosan, because they were considered unsafe, ineffective, or misbranded. Some manufacturers of antimicrobial wash products are now substituting BAC, along with benzethonium chloride and PCMX, as their main antibacterial agent, because these were not included in the banned chemicals.³⁸

Another limitation of this study was the possibility that certain chemical by-products were created after combining BAC and the conventional adhesive that could have influenced the amount and percentage of enamel demineralization. This was a confounding variable in the study that was difficult to identify and control. At present, there is no published information on this matter. Therefore, further investigation on this possibility would be beneficial.

All brackets were debonded and had to be replaced every week. A possible reason for this could have been the hard and sticky consistency of the cariogenic diet of 67% sucrose. Difficulty in isolation was also encountered during bracket bonding despite the administration of atropine sulfate to decrease salivary hypersecretion. Salivary contamination was found to considerably reduce adhesion of brackets.³⁹ Although the detected shear bond strengths of 10.12 to 20.94 MPa after incorporation of BAC in vitro¹⁶ may be sufficient to withstand the forces of mastication,¹⁹ the in-vivo bond strengths of brackets are inferior to in-vitro bond strengths.⁴⁰

Previous studies have determined the amount of enamel demineralization by visually dividing 1 or 2 tooth surfaces into 8 approximately equal areas and then scoring them subjectively.^{21,33} We used a computer program (Python programming language with Opencv library) to measure the demineralized areas using

color-based image analysis and measured 4 tooth surfaces (palatal, mesial, occlusal, and buccal). This method showed that color-based image analysis can be a more objective approach in measuring demineralized areas of enamel after murexide staining.

Our study showed that inoculation with *S sobrinus*, accompanied by a cariogenic diet, can induce enamel demineralization in rats in as early as 7 weeks, with or without orthodontic brackets.

The outcome of this study is consistent with the results of previous studies that concluded that the incorporation of 0.25% and 0.75% BAC in a composite resin confers antimicrobial effects.¹⁵⁻¹⁷ This study suggests that BAC may contribute to a decrease in demineralization. The observed decrease in enamel demineralization in subjects in the BAC group occurred not only on areas immediately surrounding the bracket, but also on other surfaces. The 0.75% BAC formulation appeared to have greater reduction in demineralization than the 0.25% BAC, particularly at the mesial area. Our sample size was too small to declare this a statistically significant result.

BAC may be effective as an antibacterial agent against *S sobrinus*, but it is still uncertain whether it will have the same clinical effect.³³ In this study, BAC was able to decrease enamel demineralization in 7 weeks; yet this is not conclusive in a clinical setting, since a patient typically undergoes several months or years of orthodontic treatment.

CONCLUSIONS

Our findings indicate that animals that received orthodontic resin with BAC showed less enamel demineralization compared with the non-BAC group. Although there was less enamel demineralization in the rats treated with 0.75% BAC than in those given

0.25% BAC in all areas, these differences were not large enough to attain statistical significance.

This study substantiates that the addition of BAC to an orthodontic composite has the potential to reduce the amount and percentage of enamel demineralization in rats. Aside from being an antibacterial agent, BAC may also have anticariogenic effects.

It is recommended that further studies on BAC be performed regarding its anticariogenic effect, effect on mechanical properties when incorporated in dental materials, shear bond strength of brackets, new products created after its combination with dental materials, cytotoxicity, and long-term systemic effects on humans. Because of the relatively large variability in size of enamel demineralization seen in this study, a study with a larger sample would be required for this purpose. An increase in sample size and testing of more concentrations of BAC are recommended.

As with the use of any antibacterial agent, the optimal and least concentration for BAC to produce an anticariogenic effect should be further studied and determined, because there is always the impending risk of bacterial resistance with its inadvertent use.

Dental caries is affected by many factors other than bacteria that must likewise be studied. Nevertheless, the use of BAC, not only in orthodontics, but also in other branches of dentistry, is worth investigating since it could reduce enamel demineralization, prevent white spot lesions, and decrease the burden of dental caries.

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