Scientific Article

Antibacterial properties of current orthodontic band cements

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Abstract

Purpose: *Manufacturers commonly provide information on the physical properties of dental materials, but information on their antibacterial properties is often missing. This study determined the antibacterial properties of four currently used orthodontic band cements against three different strains of* Streptococcus mutans.

Methods: *The cements utilized were Durelon,™ Ketac,™ Mizzy™ Zinc Phosphate, and Band-Lok,™ a recently introduced, resin-based, dual-cure glass ionomer cement. Disk diffusion assay methodology was used to test for zones of bacterial inhibition around cement samples. Zones of inhibition were measured in millimeters using an electronic caliper. In addition to cured cement plugs and freshly mixed cement samples, a new variation, in the form of a cement plug surrounding a stainless-steel band, was tested. Twelve combinations resulted from the four cement types and three forms.*

Results: *Of the variables studied, the mix forms of Durelon, Ketac, and Mizzy Zinc Phosphate cement showed the greatest bacterial inhibition (Kruskal-Wallis, $P < 0.05$). Among the cements tested, Mizzy Zinc Phosphate showed the largest zones of inhibition, with Durelon and Ketac having comparable zones of inhibition (Kruskal-Wallis, $P < 0.05$). Band-Lok did not exhibit an inhibitory effect against any of the three strains of* S. mutans *tested.*

Conclusion: *A "containment effect" of no bacterial inhibition was observed in the cement samples surrounded by the stainless-steel band material. (Pediatr Dent 20:1, 43–48, 1998)*

Fixed orthodontic appliances predispose teeth to increased plaque accumulation. Favored sites for this accumulation include cervical margins and where cement has been lost beneath bands.[1] Enamel demineralization in the form of "white spot" lesions is a negative sequela of treatment in 50% of patients undergoing orthodontic treatment.[2]

Previous studies have demonstrated that small carious lesions can be remineralized in the presence of fluoride ions.[3–5] If a cement could be shown to release fluoride, it could also have an antibacterial effect in addition to its potential remineralizing properties, and should prove useful in the clinical setting. A new dual-cure, resin-based, glass ionomer band cement, Band-Lok, has been introduced that can bond to metal as well as enamel. Its manufacturer purports that it releases fluoride, making it useful in the prevention of enamel decalcification.

The purpose of our study was to compare the antibacterial activity of four currently available orthodontic band cements: Durelon (ESPE Premier, Norristown, PA), Ketac (ESPE Premier, Norristown, PA), Mizzy Zinc Phosphate (Mizzy, Inc., Cherry Hill, NJ), and Band-Lok (Reliance Orthodontics, Itasca, IL).

In addition, a study was conducted to determine the fluoride ion release and pH changes undergone by cured samples of Band-Lok cement at 24-, 48-, and 72-h intervals.

Methods

Prior to study initiation, the primary investigator received extensive training and supervision in proper microbiological techniques and procedures. In addition, a pilot study was performed to verify the appropriateness of the study design.

Cement sample preparation

The four cements were tested in three forms generating 12 experimental sample groups as follows:

1. Cured Durelon cement plug
2. Cured Ketac Glass Ionomer cement plug
3. Cured Mizzy Zinc Phosphate cement plug
4. Cured Band-Lok cement plug
5. Cured Durelon cement plug, stainless-steel band
6. Cured Ketac Glass Ionomer cement plug, stainless-steel band
7. Cured Mizzy Zinc Phosphate cement plug, stainless-steel band
8. Cured Band-Lok cement plug, stainless-steel band

9. Durelon cement, freshly mixed
10. Ketac Glass Ionomer cement, freshly mixed
11. Mizzy Zinc Phosphate cement, freshly mixed
12. Band-Lok cement, freshly mixed

A custom, 5-mm diameter by 5-mm high polyethylene mold was fabricated to produce uniformly sized cement plug samples. Cement samples were prepared using freshly mixed cement according to the manufacturer's instructions, and cured shortly before placement in the prepared wells in the Petri dishes.

Samples of the cement and stainless-steel band materials were prepared using a custom, 5-mm diameter mold created by stock stainless-steel band material (0.125 in. x 0.004 in.), the cement occupying the center of the samples.

Each cement in the mixed sample group was prepared as above. Using sterile syringes, the uncured cement was placed into the 5-mm wells, which had been prepared within the agar and allowed to cure.

Bacterial sample preparation

S. mutans bacteria was used because of its association with dental caries.[6] Additionally, previous investigators have used *S. mutans* in similar experiments.[7,8] The following strains of *S. mutans* were used due to their classification as bacteria capable of dental caries production:

S mutans ATCC #25175
S mutans ATCC #33402
S mutans ATCC #35668

Mueller Hinton agar with 5% sheep blood was utilized as a suitable growth medium for *S. mutans*. Uniform 5-mm wells were cut within the agar to allow cement samples to touch the agar. Each plate had 5 wells made in equal thirds of the plate for a total of 15 wells per plate (Figure 1).

The Petri dishes were uniformly inoculated with one strain of *S. mutans* using the standard method for disk diffusion susceptibility to provide for a uniform bacterial lawn of growth.[9] Streaking of the plates was accomplished using a sterile, cotton-tipped applicator which had been saturated with one strain of bacteria. The applicator was drawn along the surface of the Petri dish in a zig-zag pattern to cover the entire plate surface. The Petri dish was then rotated 90° and the applicator was again drawn across the entire surface in a zig-zag fashion to ensure complete coverage of the plate. This streaking of the plates was completed following preparation of the wells.

Experimental technique

Each sample was placed in prepared wells within agar in Petri dishes that were inoculated with one of three strains of *S. mutans*. The zone of bacterial inhibition, if any, which occurred around the samples was measured from the edge of the cement sample to the edge of the zone of inhibition, to the nearest tenth of a millimeter, with an electronic digital caliper. For all samples in the study, measurements were performed three times, and averaged.

The inoculated Petri dishes and cement samples were incubated at 35°C and the zones of bacterial inhibition were measured by a single investigator at 24-, 48-, and 72-h intervals. The electronic caliper was zeroed prior to each measurement to ensure accuracy of the measurements.

In each Petri dish, five samples of one type of cement, in each of the three forms, was placed within the prepared wells, and inoculated with one of three strains of *S. mutans*; e.g. 5 Durelon plugs, 5 Durelon plugs with band, and 5 Durelon cement samples, freshly mixed, in one Petri dish inoculated with *S. mutans* #25175 (Fig 1).

pH and Fluoride release

A study of the pH changes and fluoride ion release of Band-Lok cement was made at 24-, 48-, and 72-h intervals. Five uniform samples of freshly mixed and self-cured Band-Lok cement were prepared according to the manufacturer's directions. The samples were placed in 40 mL of de-ionized water in covered polyethylene tubes, then inverted and gently agitated three times prior to pH testing with a pH electrode (Orion Research, Cambridge, MA), and fluoride release with a fluoride electrode (Orion Research, Cambridge, MA). The samples were depleted by 6 mL (2 mL for fluoride, 4 mL for pH)

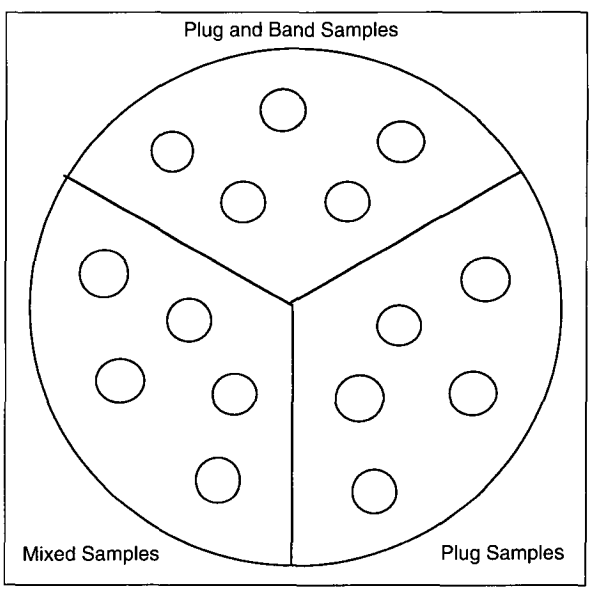

Fig 1. Experimental Petri Dish Set-Up. 5 plug, 5 plug and band, 5 mixed samples.

after each time point (incubation solution = 40 mL at 24 h, 34 mL at 48 h, 28 mL at 72 h).[10]

Statistical analysis

A Type I error of .05 and 80% power would detect a difference of 50% between the radii of the measured zones of bacterial inhibition.

As the initial data were not normally distributed, a Box Cox Transformation was completed to transform the data to a more normal distribution, resulting in raw data exponentiated to the −1.5 power.

A repeated-measures three-way ANOVA was performed with time utilized as the repeat factor (24, 48, and 72 h). The three factors utilized in the model were bacteria (ATCC #25175, #33402,and #35668), form (mix, plug, and plug with band), and cement type (Durelon, Ketac, Mizzy Zinc Phosphate, and Band-Lok).

For each bacteria type and each hour of observation, descriptive statistics were generated including means, standard deviations, medians, and ranges for the transformed data. Because it was difficult to discern whether assumptions of the three-way ANOVA were met, and as three-way interactions were significant, differences between factors—holding one or more of the other factors constant—were tested for significance using nonparametric methods.

A Kruskal-Wallis test was used to test for differences among the bacteria types for each of the time points. Similar analyses were done to assess differences among forms and cement types. For each cement type, differences among bacteria were tested for significance at each of the time intervals. For each of the forms, differences among cement types were also tested for significance at each of the time periods. For every combination of bacteria, form, and time point, differences were examined.

For purposes of illustration, means of the zones of bacterial inhibition were computed for cement types and cement forms at 24-, 48-, and 72-h time intervals and presented in tabular form.

Results

There were significant differences among the cement types, with Mizzy Zinc Phosphate having the greatest bacterial inhibition ($P < 0.05$, Kruskal-Wallis), followed by Durelon and Ketac with comparable inhibition ($P < 0.05$, Kruskal-Wallis), and Band-Lok having the least inhibition at each of the time points (Tables 1–3). There were significant differences among forms at all time points, such that the greatest bacterial inhibition occurred for the mix form and the least inhibition occurred in the plug with band form (Tables 1–3).

The effect of different microorganisms

There were no significant differences among bacteria types for the plug and band forms of cement ($P < 0.05$). Mixed cements demonstrated significantly greater inhibition against *S. mutans* #33402 ($P < 0.05$) (Table 2). Mizzy Zinc Phosphate, in the mix form, displayed significant differences at all time points com-

Table 1. Mean zones of bacterial inhibition SM#25175 (measured in millimeters ± SD)

Plug Form	*24 h*	*48 h*	*72 h*
Durelon	0	0	0
Ketac	0	0	0
Zinc Phosphate	1.33* ± 0.23	2.18* ± 0.16	2.50* ± 0.28
Band-Lok	0	0	0
Plug and Band Form	*24 h*	*48 h*	*72 h*
Durelon	0	0	0
Ketac	0	0	0
Zinc Phosphate	0.18* ± 0.15	0.31* ± 0.21	0.33* ± 0.22
Band-Lok	0	0	0
Mixed Form	*24 h*	*48 h*	*72 h*
Durelon	2.15* ± 0.18	2.18* ± 0.19	2.32* ± 0.21
Ketac	2.77* ± 0.17	3.60* ± 0.10	3.64* ± 0.10
Zinc Phosphate	3.14* ± 0.10	3.70* ± 0.17	3.72* ± 0.17
Band-Lok	0	0	0

*$P < 0.05$-Kruskal-Wallis

Table 2. Mean zones of bacterial inhibition SM#33402 (measured in millimeters ± SD)

Plug Form	*24 h*	*48 h*	*72 h*
Durelon	0.62* ± 0.08	0.64 ± 0.08	0.70 ± 0.09
Ketac	0	0	0
Zinc Phosphate	2.40* ± 0.57	3.28* ± 0.61	3.74* ± 0.66
Band-Lok	0	0	0
Plug and Band Form	*24 h*	*48 h*	*72 h*
Durelon	0.08* ± 0.06	0.09* ± 0.08	0.09* ± 0.08
Ketac	0	0	0
Zinc Phosphate	0	0	0
Band-Lok	0	0	0
Mixed Form	*24 h*	*48 h*	*72 h*
Durelon	2.84* ± 0.25	3.16* ± 0.24	3.47* ± 0.19
Ketac	1.56* ± 0.16	2.57* ± 0.23	2.75* ± 0.21
Zinc Phosphate	4.58* ± 0.30	4.52* ± 0.30	5.01* ± 0.22
Band-Lok	0	0	0

*$P < 0.05$-Kruskal-Wallis

Table 3. Mean zones of bacterial inhibition SM#35668 (measured in millimeters ± SD)

Plug Form	*24 h*	*48 h*	*72 h*
Durelon	0	0	0
Ketac	0	0	0
Zinc Phosphate	1.31* ± 0.03	1.88* ± 0.27	2.20* ± 0.40
Band-Lok	0	0	0
Plug and Band Form	*24 h*	*48 h*	*72 h*
Durelon	0	0	0
Ketac	0	0	0
Zinc Phosphate	0	0	0
Band-Lok	0	0	0
Mixed Form	*24 h*	*48 h*	*72 h*
Durelon	1.57* ± 0.05	1.63* ± 0.05	1.80* ± 0.09
Ketac	3.33* ± 0.29	3.59* ± 0.22	3.64* ± 0.21
Zinc Phosphate	3.23* ± 0.38	3.95* ± 0.57	4.07* ± 0.59
Band-Lok	0	0	0

*$P < 0.05$-Kruskal-Wallis

pared to Band-Lok, Durelon, and Ketac ($P < 0.05$) (Tables 1–3). All three strains of *S. mutans* showed the greatest inhibition with Mizzy Zinc Phosphate in the plug and mix form ($P < 0.05$) (Tables 1–3). The same organisms demonstrated the least inhibition with Band-Lok ($P < 0.05$). Band-Lok showed no inhibition in any form, regardless of the organism tested (Tables 1–3).

The effect of different cements and forms

Mixed cements demonstrated the greatest inhibition of all organisms, with Mizzy Zinc Phosphate showing the greatest inhibition and Band-Lok the least ($P < 0.05$) (Tables 1–3). Band-Lok showed no bacterial inhibition in any form (Tables 1–3). In the plug form, Mizzy Zinc Phosphate showed significant

TABLE 4. EVALUATION OF PH CHANGES AND FLUORIDE RELEASE OF BAND-LOK CEMENT

	pH changes over time		
Sample	*24 h*	*48 h*	*72 h*
1	5.07	5.78	5.80
2	5.06	5.33	5.68
3	5.05	5.52	5.59
4	4.97	5.56	5.81
5	5.30	5.69	5.88
	Fluoride release (ppm)		
Sample	*24 h*	*48 h*	*72 h*
1	0.91	1.48	1.48
2	1.30	1.71	1.85
3	1.02	1.48	1.48
4	1.50	1.71	1.80
5	0.91	1.45	1.65

Samples were inverted gently three times prior to testing for pH and fluoride release. Samples were depleted by 6 mL (2 mL for fluoride, 4 mL for pH) after each time point, (i.e., incubation solution = 40 mL at 24 h, 34 mL at 48 h, 28 mL at 72 h).

inhibition against all strains of *S. mutans* and Durelon against *S mutans* #33402 ($P < 0.05$). All but a minor inhibitory effect with Mizzy Zinc Phosphate and Durelon were blocked when cement plugs were banded with stainless-steel rings (Tables 1–3).

Fluoride release and pH alteration of Band-Lok

The mean pH for the freshly mixed Band-Lok cement samples was pH 5.09 at 24 h, which rose to a mean pH 5.75 at 72 h. The mean fluoride release for the freshly mixed Band-Lok cement samples was 1.13 ppm at 24 h, 1.57 ppm at 48 h, and 1.65 ppm at 72 h (Table 4).

Discussion

Past studies have evaluated the antibacterial properties of orthodontic cements using the disk diffusion assay method.[8, 11] Cement plugs and freshly mixed cement have been the most commonly used forms.[8, 12] An experimental design was introduced in this study; i.e., stainless-steel band material in contact with the cement sample. The goal of this combination was to more closely represent the clinical condition of a cemented orthodontic band, and to evaluate any synergistic effect of the band and cement combination. The band actually inhibited the antibacterial effect of the cements, as shown in the plug or mix form. The band apparently contains those cement components that would ordinarily diffuse and cause an inhibition. Although this containment effect may be undesirable when evaluating materials by the disk diffusion assay methodology, it more accurately approximates the clinical setting. Enamel demineralization occurs adjacent to the band/tooth interface, not just beneath the band. Orthodontic bands may limit the diffusion of low-level fluoride ions from the banded tooth and therefore reduce the fluoride ion's potential for proximalor regional remineralization.

The finding that Band-Lok cement is not inhibitory against *S. mutans* may be explained by the evaluation of fluoride release and pH changes after curing. In this aspect of the study, the lowest pH recorded was 4.97 with 5.09 the mean pH at 24 h. The pH continued to rise during the next 48 h with a mean pH of 5.75 at 72 h. A pH of at least 4.0 has been reported as necessary for a cement to exhibit a bactericidal effect.[8] In DeSchepper and coworkers' study,[8] all antibacterial activity of the cements studied was lost when the pH of the liquid components was adjusted to 5.0.

The minimum range of fluoride release to inhibit *S. mutans* ranges from 20 to 300 ppm.[13] In this study, very low levels of fluoride release were recorded during the 72-h period of evaluation period following the curing of samples. At 24 h, the highest recorded level was only 1.50 ppm, with a mean of 1.13 ppm. At 72 h, the highest level recorded was 1.85 ppm, with a mean of 1.65 ppm.

When evaluating an antibacterial effect with the disk diffusion assay methodology, diffusion of inhibitory substances is required. A possible explanation for the lack of an inhibitory effect may lie with the chemical composition of Band-Lok. As a resin-based, glass-ionomer cement, substances which may have provided an inhibitory effect could have been chemically bound and unable to diffuse.

Band-Lok cement appears inferior to other commonly used orthodontic band cements (i.e., Durelon, Ketac, and Mizzy Zinc Phosphate) from the standpoint of being inhibitory to *S. mutans*. However, Band-Lok cement could prove to be clinically useful if it were shown to be less soluble in the oral environment than the other cements. If cement wash-out was completely eliminated, then decalcification beneath the band may not be an issue, due to the exclusion of acid-producing bacteria. An inhibitory bacterial effect at the tooth/cement margin would still be highly desirable in the prevention of enamel decalcification, secondary to bacterial colonization. Studies should be conducted to evaluate the relative solubility of Band-Lok cement.

In an attempt to increase the antibacterial effect of Band-Lok cement, the manufacturer could consider

modifications of its composition to increase the amount of fluoride released by the cement following curing. Lowering the pH of the cement, which would also increase its antibacterial effect, would most probably be ill-advised, due to the potential for enamel decalcification shown with cements exhibiting a low pH on setting.[15]

Mizzy Zinc Phosphate, Durelon, and Ketac do have significant antibacterial properties against the three strains of *S. mutans* shown to cause dental caries. These results agree with previous studies that showed the greatest bacterial inhibition occurring with cements containing zinc oxide or release fluoride.[11,16] The finding that the freshly mixed form of the cements showed the most bacterial inhibition has also been shown by previous studies.[7,8]

Other researchers have used bacteria such as *Lactobacillus salivarius*, *Streptococcus sobrinus*, and *Actinomyces viscosus*, in addition to *S. mutans*, in an attempt to more accurately duplicate the microbial conditions of the mouth.[7,11] *S. mutans* was chosen because of its proven role in the production of dental caries.[6,17] The mouth is a complicated microbiological ecosystem that is virtually impossible to duplicate in an experimental design, because of the multitude of synergistic bacterial interactions involved in the production of dental caries.

Since the completion of this study, Ultra Band-Lok has been introduced by Reliance Orthodontics. Ultra Band-Lok is similar to Band-Lok in that it is a resin-based, glass-ionomer cement, but differs in that it is exclusively a light-cured cement whereas Band-Lok is a dual-cure cement. According to the manufacturer, the fluoride release of the two products are similar.[16]

Conclusions

1. The orthodontic band's containment effect tends to limit any antibacterial properties of the cements tested
2. Band-Lok cement does not exhibit an antibacterial effect against three strains of *S. mutans* tested
3. The pH changes and fluoride release of Band-Lok cement appear insufficient to be bacteriostatic
4. The mix form of Mizzy Zinc Phosphate, Durelon, and Ketac cements exhibited the greatest bacteriostatic properties compared with other forms
5. Mizzy Zinc Phosphate is the most bacteriostatic, with Durelon and Ketac cement having comparable bacteriostatic properties.

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