ORIGINAL ARTICLE

Antibacterial properties of 4 orthodontic cements

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Background: White spot lesions are observed in nearly 50% of patients undergoing orthodontic treatment. Long-lasting antibacterial properties of orthodontic cements can reduce this phenomenon. **Methods:** The antibacterial properties of 4 orthodontic cements were evaluated by direct contact test (DCT) and agar diffusion test (ADT). With the DCT technique, octet specimens of glass ionomer (CX-Plus; Shofu, Kyoto, Japan), reinforced glass ionomer (GC Fuji ORTHO LC; GC Corporation, Tokyo, Japan), and 2 composite (Transbond XT and Transbond Plus; 3M Unitek, Monrovia, Calif) orthodontic cements were placed on the sidewalls of wells of a 96-microtiter plate. *Streptococcus mutans* cells (ca. 1×10^6) were placed on the surface of each specimen for 1 hour at 37°C. Then, fresh media was added to each well, and bacterial growth was monitored for 16 hours with a temperature-controlled spectrophotometer. This was repeated on specimens aged in phosphate-buffered saline for 1 day, 1 week, and 1 month. The ADT was performed by placing specimens in wells punched in agar plates. **Results:** Measurement of the halo in bacterial lawn after 48 hours showed that only the glass ionomer cement (CX-Plus) produced an inhibition zone (1.2 mm around the sample). Results at the DCT showed that only the reinforced glass ionomer cement (GC Fuji ORTHO LC) exhibited potent antibacterial activity, which lasted 1 week and diminished over the next 3 weeks. **Conclusions:** The reinforced glass ionomer cement possessed the most potent and long-lasting antibacterial activity. (Am J Orthod Dentofacial Orthop 2005;127:56–63)

Fixed orthodontic appliances predispose teeth to accumulate plaque, mainly at the cervical margins of bands and brackets.[1] These devices are attached to the teeth by various dental cements, adhesive resins, and hybrid cement–resin combinations that offer improved physical properties and clinical benefits.[2] Glass ionomer cements possess low-strength chemical binding to enamel, are brittle, and fracture cohesively, whereas the technique-sensitive resin adhesives provide higher mechanical retention to etched enamel. Hybrid materials combine the advantages of cements and resins without overcoming certain disadvantages, including solubility and shrinkage.[3] Most currently available orthodontic cements are not ideal in their handling, manipulation, or clinical performance.

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Initial caries, in the form of white spot lesions, is a negative treatment sequela observed in 50% of patients undergoing orthodontic treatment.[4]

This enamel demineralization is principally *Streptococcus mutans*–associated disease. Therefore, it is important to evaluate the interaction of orthodontic cements with these bacteria, including effect on adhesion, bacterial viability, and biofilm formation.[5-9]

Agar diffusion test (ADT) was the popular assay for antibacterial properties used in most of these studies, despite its acknowledged limitations, which include its semiquantitative nature, ability to measure only the activity of soluble components, and difficulties in controlling some variables (ie, density of bacterial inocula, growth medium, agar viscosity, storage conditions of agar plates, size and number of specimens per plate, contact between specimens and adjacent agar, and incubation time and temperature).[10]

The direct contact test (DCT) measures quantitatively the effect of direct and close contact between the test microorganism and the tested materials, regardless of the solubility and diffusibility of their components.[11]

We evaluated the antibacterial properties of 4 different orthodontic cements, using both the ADT and the DCT. The cement–bacteria interaction was evaluated in fresh and aged specimens.

MATERIAL AND METHODS

The following orthodontic cements were tested in this study: reinforced glass ionomer cement (GC Fuji ORTHO LC; GC Corporation, Tokyo, Japan); conventional glass ionomer cement (CX-Plus; Shofu, Kyoto, Japan); and 2 composite resin cements (Transbond XT and Transbond Plus; 3M Unitek Dental Products, Monrovia, Calif). *S. mutans*, the primary etiologic agent of caries and a frequent caries-lesion isolate, was used as the test microorganism. *S. mutans* has been used widely for testing antimicrobial activity of restorative materials.[12]

S. mutans 23571M was grown aerobically from frozen stock cultures in brain heart infusion (BHI) broth containing 0.5% bacitracin at 37°C. Bacteria were used at their late-logarithmic to early-stationary phase.

The ADT was performed on mitis salivarius agar plates. Each plate was inoculated with 200 μL of freshly grown *S. mutans* (OD 0.6 at 650 nm). Eight 4-mm-diameter holes were punched in the agar surface of each plate, and the respective orthodontic cement was introduced and polymerized immediately. The plates were incubated at 37°C for 48 hours and then visually inspected for the presence of inhibition zones in the bacterial lawn. Bacterial inhibition zone halo was measured in 2 perpendicular locations and expressed in millimeters. The ADT for each material was repeated 4 times.

The DCT[11] was based on turbidometric determination of bacterial growth in 96-well microtiter plates (96-well, flat-bottom Nunclon; Nunc, Copenhagen, Denmark). In 8 wells, the sidewall was coated evenly with a measured amount of the tested material while the plate was held vertically (ie, the plate's surface was perpendicular to the floor). A thin coat was applied with a small, flat-ended dental spatula. The material was allowed to set, or it was light polymerized in strict compliance with the manufacturers' recommendations. Special care was taken to prevent the material's flow to the bottom of the well, which would interfere with the light path through the microplate well. A 10-μL bacterial suspension (0.9-1.1 $\times$ 10^6 colony-forming units, calculated from viable counts performed separately for each experiment) was placed on the test material while the plate remained vertical. Evaporation of the suspension's liquid ensured direct contact between the bacteria and the tested materials; this usually occurred within 1 hour at 37°C. Then, BHI broth with 25 μg/mL bacitracin (220 μL) was added to each of the wells and gently mixed for 2 minutes. Eight uncoated wells in the same microtiter plate served as positive control (ie, identical bacterial inoculum was placed on the sidewall of the uncoated wells and processed as in the experiment wells). The negative control consisted of a set of wells coated with the tested materials, containing equal volumes of uninoculated medium. The kinetics of the outgrowth in each well was recorded at 650 nm every 30 minutes for 16 hours with a temperature-controlled spectrophotometer set at 37°C (VERSAmax; Molecular Device Corporation, Menlo Park, Calif). Auto-mixing before each reading ensured a homogeneous bacterial cell suspension. The experimental setup is shown in Figure 1. The linear portion of the growth curve, which correlates with bacterial growth rate, was expressed as a linear mathematical formula. Analysis of variance (ANOVA) and Tukey multiple comparison procedures (SPSS for Windows version 11.0; SPSS, Chicago, Ill) were applied on the slopes of these linear formulas.[13]

Similar experiments were performed in which the tested materials were aged for 1 day, 1 week, and 1 month. Aging was performed with phosphate-buffered saline (PBS) containing 25 μg/mL bacitracin, which was replaced every 48 hours.

Parallel to the experimental setup, calibration experiments were performed in each plate to establish the bacterial growth rate for each experiment; this allowed the comparison of data between plates. For this purpose, 10 μL of bacterial suspension (ca. 10^6cells) was placed on each sidewall of 3 wells in a 96-well microtiter plate, as in the experimental setup. Then, 275 μL of fresh medium was added and the plate gently mixed for 2 minutes. From each well, 55 μL were transferred to an adjacent set of wells that contained 220 μL of fresh medium. This procedure was repeated 8 times.

RESULTS

Performance of the ADT on 8 specimens of CX-Plus showed an inhibitory halo in the bacterial lawn of 6.4 $\pm$ 0.51 mm; no inhibitory halos around specimens of the 3 other cements were observed.

To maintain the quantitative nature of the DCT, a calibration growth curve was performed in each experiment. For this purpose, bacteria were diluted by a factor of 5 (Fig 2); each point on the curve is the average of 3 wells measured at the same time.

DCT was performed on 8 specimens of each of the 4 materials tested. A regression line was performed on the linear segment of the curve, which represents the logarithmic phase of growth. The R^2 of all growth curves ranged from 0.99 to 0.94. Two-way ANOVA, performed on all experiments, indicated a significant difference in bacterial growth rate (slope) between the cements in a combination of time and material ($P <$ .001).

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