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Surface antibacterial properties of glass ionomer cements used in atraumatic restorative treatment

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Atraumatic restorative treatment (ART), previously known as alternative restorative treatment,[1] originally was developed to provide restorative dental treatment outside the traditional clinical setting. Its use has increased in the last few years. This approach for dental caries treatment was developed around 1995 and involves removing soft, demineralized tooth tissue using only hand instruments. The tooth then is restored with an adhesive restorative material, usually glass ionomer. This treatment is recommended by the World Health Organization, and it offers significant advantages (such as provision of restorative dental treatment outside the dental office setting, a biologically friendly approach, minimal cavity preparations, high level of survival and low costs) to populations in developing countries that have difficulties accessing or have no access to dental care.[2-6]

The selection of an appropriate restorative material often is dictated by the compromised conditions of the cavity preparation. High survival rates in both primary and permanent dentitions have been reported in single-surface ART restorations that use high-viscosity glass ionomer (range, 95-97 percent after one year to 86-72 percent after three-six years).[7] Most of the pub-

A B S T R A C T

Background. Atraumatic restorative treatment (ART) is recommended for use worldwide, not only in developing countries where resources are not readily available, but also in more industrialized countries. The antibacterial properties of restorative dental materials may improve the restorative treatment outcome. Glass ionomer cement (GIC) has been advocated as the preferred restoration material for ART. The authors evaluated the antibacterial properties of restorative materials— three GICs and a zinc oxide eugenol (ZOE)—in vitro.

Methods. *Streptococcus mutans, Actinomyces viscosus* and *Enterococcus faecalis* were the test microorganisms. The authors used a quantitative microtiter spectrophotometric assay to evaluate the antibacterial effect of the restorative materials using the direct contact test (DCT) of freshly prepared and one-week-aged materials.

Results. The freshly prepared GICs and ZOE showed no bacterial growth in all tested bacteria compared with a control. This effect lasted for at least one week for *S. mutans* and *A. viscosus* but not for *E. faecalis*.

Conclusions. Conventional GICs used in ART showed antibacterial surface properties against cariogenic bacteria for at least one week. Further study on the long-term antimicrobial effects of GICs is needed.

Clinical Implications. The antimicrobial properties of freshly prepared restorative materials and aged restorative materials used in ART have a potent effect against cariogenic bacteria. These properties have crucial importance in preventing secondary caries.

Key Words. Glass ionomers; bacteria; atraumatic restorative treatment.

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lished reports about ART have focused on the physical properties of glass ionomer cements (GICs), and the effectiveness and longevity associated with using ART with GIC.[3,7,8] There is a paucity of information in the literature regarding their antibacterial effect in the use of ART.

Although restorative materials with long-lasting antibacterial surface properties may reduce the biofilm and, thus, caries recurrence, the importance of the antibacterial effect of ART and its caries preventive effect via microflora change has been addressed only as early as 2003.[9] Most dental restorative materials do not have a long-lasting, perfect seal with the restoration wall, which can lead to leakage of oral fluids and a percolation effect, followed by bacterial penetration and growth.[10,11] A few studies have suggested that practitioners use GICs containing chlorhexidine to inhibit bacteria associated with caries for both affected and infected dentin.[12-14]

The antibacterial effect of many dental restorative materials has been examined by using the agar diffusion test[15,16] or by testing the material's minimum inhibitory concentration.[17-22] These methods are based on measuring water-soluble components released from the bulk of the materials, and they often are used to evaluate antibiotics. The suitability of these methods for testing restorative materials, which are intended to last in an aqueous environment for many years, is questionable.

We conducted a study to evaluate the surface antibacterial effect of conventional GICs used in ART on *Streptococcus mutans, Actinomyces viscosus* and *Enterococcus faecalis.*

MATERIALS AND METHODS

Bacteria and growth conditions. We used *S. mutans* (American Culture Type Collection no. 27351), *A. viscosus* (American Culture Type Collection no. 43146) and *E. faecalis,* which was streptomycin-resistant[23] and originally isolated from human dental plaque. *S. mutans* and *A. viscosus* have been found to be associated frequently with caries.[24,25] *E. faecalis* has been shown to be a highly resistant bacteria in the root canal system, and it plays an important role in endodontic treatment failures.[23]

We cultured the bacteria aerobically overnight at 37°C in 5 milliliters of brain-heart infusion (BHI) broth (BHI, Difco, Detroit). We transferred the top 4 mL of the resulting undisturbed bacterial cultures to new test tubes and centrifuged

them for 10 minutes at 3,175 gravity. We discarded the resulting supernatant, resuspended the bacteria in 5 mL of phosphate-buffered saline (PBS) with a pH of 7.5 (Sigma-Aldrich, St. Louis) and mixed it gently by vortexing it for 10 seconds. We diluted 800 microliters of the cultures to 10^6 cells/mL.

To minimize contamination, we added 62.5 milligrams per milliliter of the antibiotic Bacitracin (Sigma-Aldrich) to the BHI broth and PBS for *S. mutans* and 5 mg/mL to the BHI broth and PBS of streptomycin for *E. faecalis*. In experiments performed with *A. viscosus,* we verified a lack of contamination through microscopic examination.

Materials tested. We tested the antibacterial properties of three commercially available GICs used in ART: Fuji IX GP (GC America, Alsip, Ill.), Fuji Plus (GC America), Ketac Molar (3M ESPE Dental AG, Seefeld, Germany). We prepared the GICs in strict compliance with the manufacturers' recommendations. We used zinc oxide eugenol (ZOE) (IRM, Dentsply Caulk, Milford, Del.) as a control material.

Direct contact test (DCT). We used the DCT to test the antibacterial properties of the GIC and ZOE as described previously[17-19]; we positioned a 96-well, flat-bottomed microtiter plate (Nunclon, Nunc, Copenhagen, Denmark) vertically. We coated eight wells with the tested material samples by applying the material to the sidewalls using a flat-ended dental spatula to ensure a uniform surface area.

We mixed the GIC samples according to the manufacturers' instructions, and they self-polymerized. We placed 10 µL of the bacterial suspension on each sample in a set of eight wells and incubated the plate in a vertical position for one hour at 37°C. During that time, most of the suspension liquid evaporated, ensuring direct contact between all bacteria and the tested material surface. Then we added 220 µL of BHI broth to each well and placed the plate in a temperature-controlled microplate spectrophotometer (VersaMax, Molecular Devices, Sunnyvale, Calif.) set at 37°C. We estimated the bacterial outgrowth after direct contact with the tested material on the basis of the changes in the read-

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ABBREVIATION KEY. ART: Atraumatic restorative treatment. **BHI:** Brain-heart infusion. **DCT:** Direct contact test. **GIC:** Glass ionomer cement. **PBS:** Phosphate-buffered saline. **ZOE:** Zinc oxide eugenol.

ings of optical density at 650 nanometers that were recorded by the spectrophotometer every 20 minutes for 16 hours. The spectrophotometer mixed the samples for five seconds before each reading. We repeated the experiments three times.

We plotted the absorbance measurements to provide bacterial growth curves for each well in the microtiter plate. We transferred the linear portion of the curve, which correlated with bacterial growth rate, and expressed it as a linear mathematical formula. We conducted analysis of variance and a Tukey multiple comparisons procedure and applied them on the slope of these linear formulas. The level of significance was $P < .05$.

We conducted similar experiments after aging the tested materials for seven days at 37°C in the presence of PBS, which was replaced every 48 hours. In each microtiter plate, a set of eight wells served as the control; bacteria grew on microtiter sidewalls that were not coated with any of the tested materials. We tested an additional set of eight wells in which each tested material was processed as above in sterile conditions without any bacteria.

RESULTS

S. mutans growth in a 96-well microtiter plate is shown in Figure 1. (Points on the curve of graphs represent the mean values measured in the eight wells containing the same tested material.) The standard deviation of the measurements did not exceed 7 percent of the absolute values.

The three GICs and the ZOE showed no bacterial growth when compared with the control (Figure 1A). The growth curves the spectrophotometer recorded for the freshly prepared GIC samples were similar to each material's sterile sample (data not shown). Changes in the optical density in all three GIC sterile samples did not mimic the logarithmic curve seen in the samples from the wells that contained bacteria; this likely was due to interaction with the BHI broth. This view was supported by the fact that we did not see similar changes in optical density with the
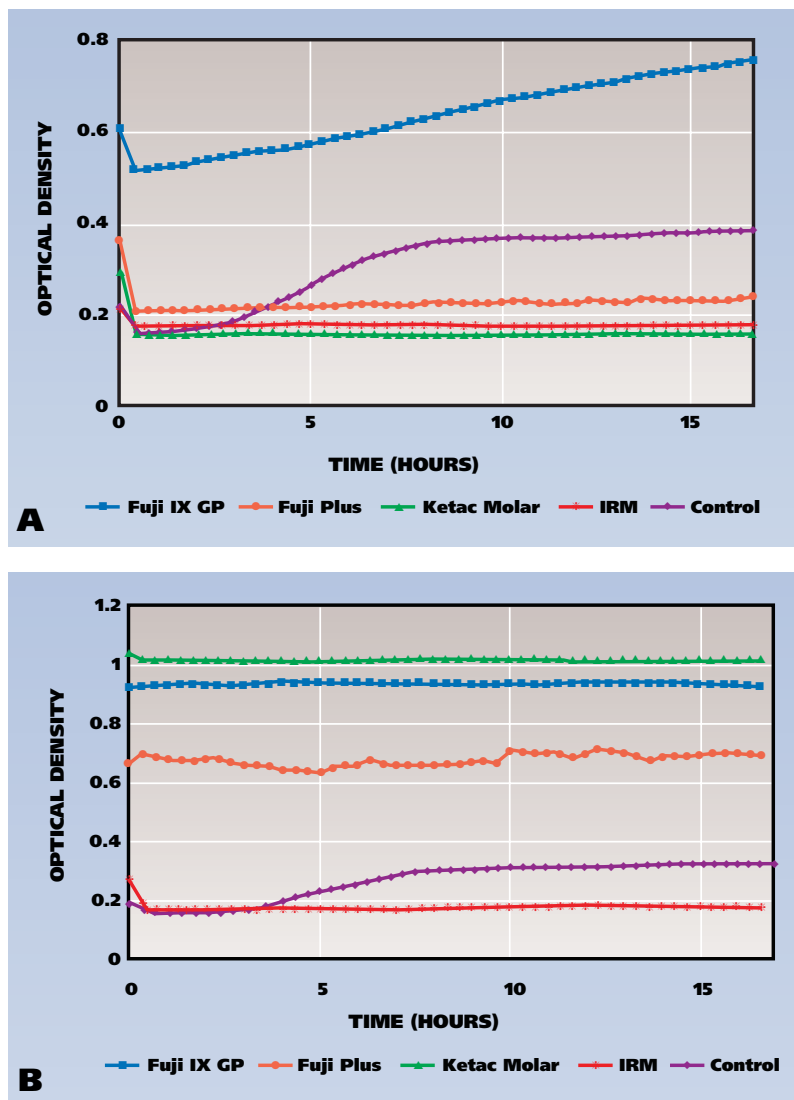
Figure 1. Growth curves of bacterial outgrowth after direct contact between *Streptococcus mutans* and four freshly prepared dental restorative materials **(A)** and the same four materials aged for one week **(B)**, as well as the control. Fuji IX GP is manufactured by GC America, Alsip, Ill. Fuji Plus is manufactured by GC America. Ketac Molar is manufactured by 3M ESPE Dental AG, Seefeld, Germany. IRM is manufactured by Dentsply Caulk, Milford, Del.

aged materials (Figure 1B), which had been exposed to recurrent replacement of the PBS. The fact that the Fuji IX GP samples inoculated with *S. mutans* had growth curves similar to the sterile sample implies that there was no bacterial growth.

Bacterial growth curves for aged samples in similarly prepared microtiter plates showed no growth of *S. mutans* in direct contact with all tested materials' surfaces as compared with the control (Figure 1B). The growth curves recorded for Fuji IX GP, Fuji Plus and Ketac Molar were similar to those of the sterile samples, indicating
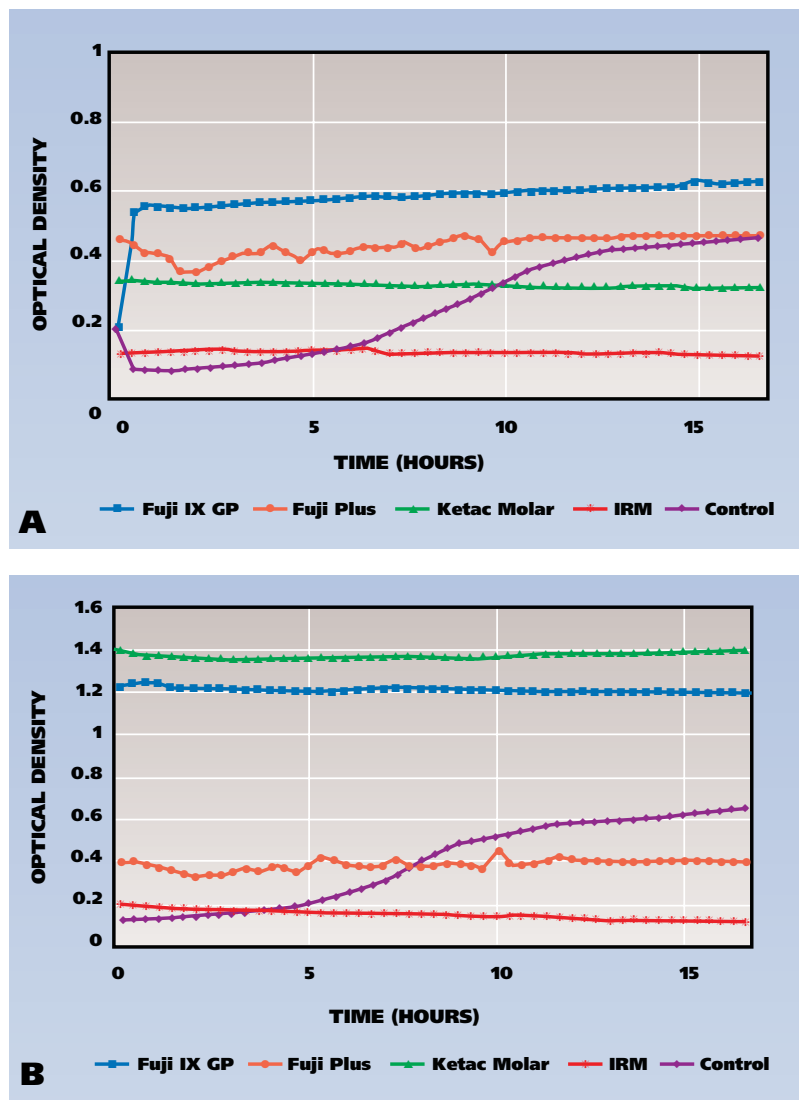
Figure 2. Growth curves of bacterial outgrowth after direct contact between *Actinomyces viscosus* and freshly prepared dental restorative materials **(A)** and the same four materials aged for one week **(B)**, as well as the control. Fuji IX GP is manufactured by GC America, Alsip, Ill. Fuji Plus is manufactured by GC America. Ketac Molar is manufactured by 3M ESPE Dental AG, Seefeld, Germany. IRM is manufactured by Dentsply Caulk, Milford, Del.

that the curves depicted the materials' behavior in BHI broth and not bacterial growth.

As in the *S. mutans* group, only the control samples in the *A. viscosus* group showed logarithmic growth. The tested material samples showed no logarithmic growth, either in the freshly prepared samples (Figure 2A) or in the aged samples (Figure 2B).

The GIC and the ZOE samples in the *E. faecalis* group showed no bacterial growth in any of the freshly prepared samples compared with the control samples (Figure 3A). However, logarithmic bacterial growth was recorded after direct

contact with the aged samples, except in the ZOE samples, for which no bacterial growth was detected (Figure 3B).

DISCUSSION

GIC restorative materials have advantages such as the ability to bond chemically to enamel and dentin, biocompatibility with pulpal tissue, good cavity seal, ease of use and low costs.[26,27] GIC restorative margins have been found to have lower levels of *S. mutans* and plaque, which suggests that plaque formed on GIC restorations has less potential to induce recurrent caries.[28,29] Inhibition of enamel demineralization immediately adjacent to GIC restorative margins has been found.[6] Studies link the antibacterial effect to fluoride ion release, which reduces the plaque's acidogenicity that does not favor *S. mutans*.[26-27,30] The fluoride release from GICs is greatest in the first few days after placement, after which time it decreases to a constant level over a prolonged period.[28-30]

In our study, we used a quantitative in vitro test to analyze the surface antibacterial properties of GICs used in ART as compared with ZOE on different oral bacteria. In DCT, bacteria are allowed to come in direct contact with tested material under controlled conditions. We used the PBS replacement in the aging process to mimic exposure of the tested materials to an aqueous environment, since the effect of extracting soluble products such as fluoride ions from GICs may reduce the inhibitory effect of the material significantly. We found that all three GICs completely inhibited the growth of *S. mutans*, *A. viscosus* and *E. faecalis*. This effect lasted for at least one week in *S. mutans* and *A. viscosus,* both of which are cariogenic bacteria. Only *E. faecalis* showed logarithmic bacterial growth after direct contact with the aged materials. This may be attributed to the resistant character of *E. faecalis*. These findings support reports of lower counts of microorganisms in the margins of GIC restorations.[6,28,29] The reduced bacterial growth after direct contact with the GIC may be related to the fluoride release as described elsewhere.[28]

In our study, we compared the antibacterial

properties of three GICs with those of a ZOE (a possible filling material in field conditions). The GICs we tested—Fuji Plus, Fuji IX GP and Ketac Molar—had similar optical density readings in both the test and the sterile samples. These readings may depict the inherent behavior of the materials in an aqueous environment; thus, it may be assumed this phenomenon is a depiction of the GICs dissolution behavior.[2,3,31] The changes in the sterile samples' curves were steeper for the freshly prepared materials than for the aged materials. This can be attributed to aging the samples in PBS, which was replaced every 48 hours, allowing the dissolved products to be washed away.

From a clinical standpoint, the fluoride release of the GICs may drop significantly with long-term usage as reported in other studies.[29,32] However, it is not known whether the fluoride release levels remain effective or for how long. Further studies should be conducted to examine the long-term antibacterial effect of GICs and the levels of fluoride release.

CONCLUSIONS

The three GICs used in this study demonstrated potent antibacterial effects against pure strains of *S. mutans*, *A. viscosus* and *E. faecalis* under DCT. This effect was lost for GICs aged one week in the case of *E. faecalis*. Whether these findings have clinical relevance requires further investigation. ∎

Figure 3. Growth curves of bacterial outgrowth after direct contact between *Enterococcus faecalis* and four freshly prepared dental restorative materials **(A)** and the same four materials aged for one week **(B)**, as well as the control. Fuji IX GP is manufactured by GC America, Alsip, Ill. Fuji Plus is manufactured by GC America. Ketac Molar is manufactured by 3M ESPE Dental AG, Seefeld, Germany. IRM is manufactured by Dentsply Caulk, Milford, Del.

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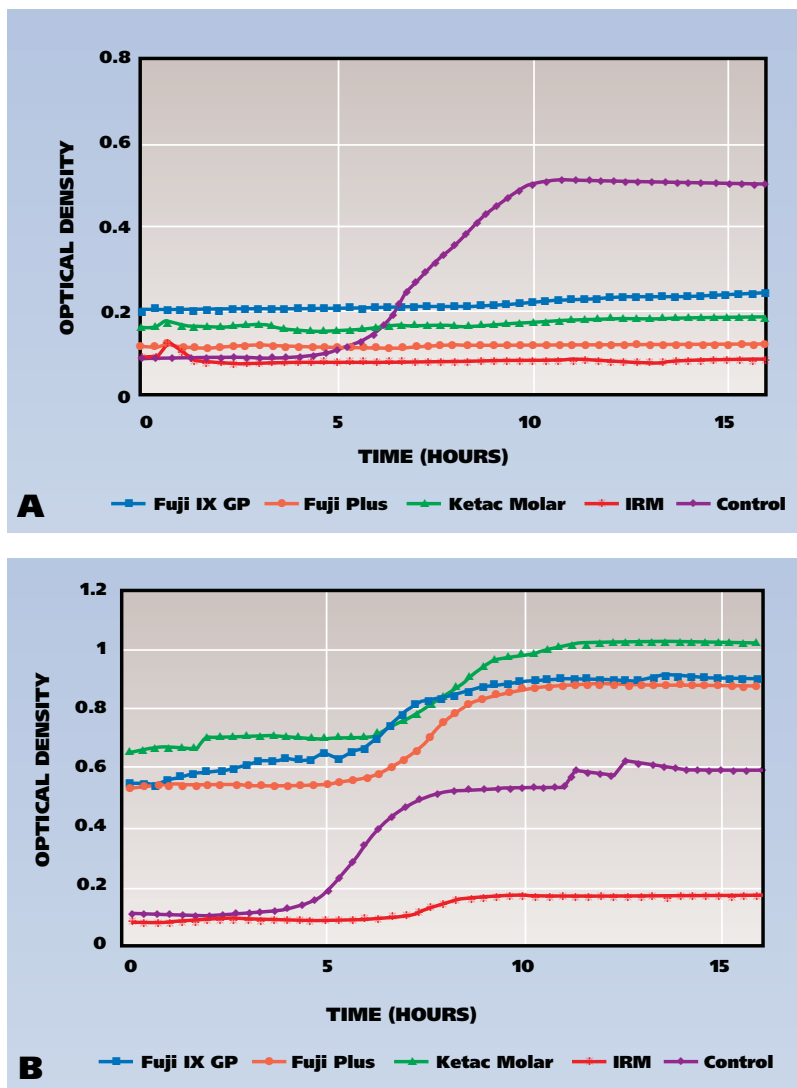
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