Surface antibacterial properties of resin and resin-modified dental cements

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Objective: Cements possessing antibacterial properties may reduce bacteria-induced fixed partial denture complications. The purpose of this study was to evaluate the antibacterial properties of 4 dental cements using the direct contact test (DCT) and the agar diffusion test (ADT). **Method and Materials:** The ADT was performed using mitis salivarius agar plates. Each plate was evenly inoculated with freshly grown mutans streptococci. Two samples of each test material—RelyX ARC, Variolink II, GC FujiCEM, and Principle—were placed, and the inhibition halo obtained was measured after 48 hours. For the DCT, 8 samples were placed on the sidewalls of wells in a 96-well microtiter plate. After polymerization, freshly grown *Streptococcus mutans* cells (1×10^6) were placed on the surface of each sample for 1 hour at 37°C. Fresh medium was then added to each well, and bacterial growth was followed for 16 hours in a temperature-controlled spectrophotometer. Similarly prepared samples were aged in phosphate-buffered saline for 1 or 7 days and the DCT was repeated. Analysis of variance (ANOVA) and Tukey multiple comparisons were applied to the data. **Results:** In the ADT, GC FujiCEM showed an inhibition zone of 2.0 ± 0.3 mm, and Principle showed an inhibition zone of 1.2 ± 0.2 mm. In the DCT, freshly polymerized samples of GC FujiCEM and Principle exhibited potent antibacterial properties, while samples of Variolink II and RelyX ARC showed moderate antibacterial properties. Principle showed some antibacterial properties even after 1 day ($P < .001$). **Conclusion**: None of the tested cements in this study possesses long-term antibacterial properties. *(Quintessence Int 2007;38:55–61)*

Key words: agar diffusion test, antibacterial, dental cements, direct contact test, *Streptococcus mutans*

On the strenuous route to the perfect tooth-supported fixed partial denture (FPD), cementation is one of the crucial elements.

Cements can be divided into 5 main groups: *(1)* zinc phosphate cement, *(2)* polycarboxylate cement, *(3)* glass-ionomer cement, *(4)* resin cement, and *(5)* resin-modified glass-ionomer cement. Traditionally cements have been used to fill gaps between the tooth structure and the FPD, thereby providing increased retention.[1] To fulfill this role, cements are required to be insoluble and preferably quick-setting materials. Novel cements are expected to adhere to the tooth structure, on the one hand, and to a variety of materials used for FPDs, on the other. Of the previously mentioned groups of cements, only resin cement, resin-modified glass-ionomer cement and, to some extent, glass-ionomer cement can adhere both to the FPD and to the tooth structure.[2–5] Cement also serves as a sealing agent along the finishing line of the FPD, thereby preventing bacteria and oral fluid

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leakage, which may result in bacteria-induced complications in FPDs.[6–9]

The antibacterial properties of cements and restorative materials have been evaluated in vitro using various methodologies.[10–14] The agar diffusion test (ADT) served as the standard assay in most of these studies, despite its known limitations. Problems associated with the ADT include its qualitative nature, ability to measure only soluble components, inability to distinguish between bacteriostatic and bactericidal effects, and difficulties in comparing a large number of samples and in controlling a large number of variables.[15]

Weiss et al[16–21] introduced the direct contact test (DCT), which quantitatively measures the effect of direct and close contact between the test microorganism and the tested materials, regardless of the solubility and diffusiveness of their components.

Lewinstein et al[21] demonstrated that Duralon (3M Espe), a polycarboxylate cement, and Harvard cement (Harvard Dental), a zinc phosphate cement, possessed prolonged antibacterial properties, while KetacCem (3M Espe), a glass-ionomer cement, exhibited no antibacterial activity.

The purpose of this study was to investigate the antibacterial properties of cements: 2 resin cements, a resin-modified glass-ionomer cement, and a compomer cement, not investigated by Lewinstein et al.[21]

METHOD AND MATERIALS

Materials

The following commercially available cements were tested: 2 resin cements, RelyX ARC (3M Espe) and Variolink II (Vivadent); a resin-modified glass-ionomer cement, GC FujiCEM (GC); and a compomer cement, Principle (Dentsply DeTrey). The materials were mixed and/or polymerized in strict compliance with the manufacturers' instructions.

Test microorganism and growth conditions

Streptococcus mutans is the primary etiologic agent of caries, and therefore it is commonly used to test the antibacterial properties of restorative materials.[10–14,16–22] *S mutans* 27351M was clinically isolated on mitis salivarius agar plates. *S mutans* was grown aerobically from frozen stock cultures in brain-heart infusion (BHI) broth (Difco Laboratories) containing 8 μg/mL of Bacitracin (Merck) for 48 hours at 37°C before use.

Experimental design

Two methods were used in this study: the qualitative ADT and the quantitative DCT.

ADT. The ADT was performed using mitis salivarius agar plates. Each plate was inoculated with 200 μL of freshly grown mutans streptococci (optical density [OD] $_{650\ nm}$ = 0.5) evenly spread with a Dragalsky glass stick. To test duplicate samples, 8 holes, 4 mm in diameter, were punched in the agar. Each chemically polymerized test material was mixed and immediately introduced into each punched hole, completely filling it. The light-polymerized test materials were similarly introduced, completely filling the punched holes, and then polymerized with the aid of a curing light unit (VIP, Bisco). After incubating at 37°C for 48 hours, the plates were inspected for the presence of an inhibition zone. Two perpendicular measurements of the inhibition halo diameter were made with a caliper. The ADT was repeated in triplicate.

DCT. The DCT, described by Weiss et al[16–21] (Fig 1), is based on determination of bacterial growth in a 96-well microtiter plate (96-well flat-bottom, Nunclon, Nunc). The outgrowth kinetics in each well was recorded continuously by measuring the optical density at 650 nm every 30 minutes for 16 hours, using a temperature-controlled spectrophotometer set at 37°C (VesaMax, Molecular Device Corporation).

A microtiter plate was held vertically, with the plate surface perpendicular to the floor, and the sidewall of 8 wells was coated evenly with a measured amount of the test material, at a surface size of 19.67 ± 0.03 mm (designated as group A). A thin coat was achieved by using a small flat-ended dental instrument, such as a dental spatula. Each chemically polymerized test material was mixed and immediately introduced to the well. The light-polymerized test materials were similarly introduced and then polymerized

with the aid of a curing light unit. The materials were allowed to set in compliance with the manufacturers' recommendations. Special care was taken to prevent flow of the materials to the bottom of the well, which could interfere with the light path through the microplate well and result in false readings.

A 10-μL bacterial suspension (approximately 10^6 bacteria) was placed on the test material, while the plate remained in a vertical position. Wells were inspected for evaporation of the suspension's liquid, ensuring direct contact between bacteria and the test materials. This usually occurred within 1 hour at 37°C. BHI broth supplemented (235 μL) with Bacitracin was added to each of the group A wells and gently mixed (Gyrotory Shaker, New Brunswick Scientific) for 2 minutes; 15 μL were then transferred from group A wells, respectively, to an adjacent set of 8 wells containing 205 μL fresh medium (designated as group B). This resulted in 2 sets of 8 wells for each test material containing an equal volume of liquid medium (220 μL), so that bacterial outgrowth could be monitored and compared, both in the presence and in the absence of the test material.

One set of 3 uncoated wells in the same microtiter plate served as the positive control. Identical bacterial inoculum was placed on the sidewall of these uncoated wells and processed as in the experimental groups.

The growth curves from both experimental groups were compared with the control outgrowth. The negative control consisted of a set of 4 wells coated with the test materials as in experimental group A containing an equal volume of uninoculated fresh medium. The kinetics of the outgrowth in each well was monitored at 650 nm at 37°C and recorded every 30 minutes for 16 hours using the same temperature-controlled microplate spectrophotometer. Automixing prior to each reading ensured a homogeneous bacterial cell suspension. Data were recorded in optical density units.

Parallel to the experimental design, calibration experiments were performed to establish bacterial outgrowth under experimental conditions in a quantitative and reproducible manner. In a typical experiment, 10 μL of bacterial suspension (approximately 10^6 cells) was placed on each sidewall of 3 wells in a 96-well microtiter plate, as in the experimental design. Fresh medium, 285 μL, was added and the plate gently mixed for 2 minutes. From each well, 55 μL were transferred to an adjacent set of wells, respect-ively, that contained 230 μL fresh medium. The dilution transfer was repeated 6 consecutive times and the plate gently remixed for 2 minutes. To establish the number of viable bacteria, 10 μL was transferred from each dilution to a mitis salivarius agar plate. The plates were incubated at 37°C for 48 hours. The number of colony-forming units (CFUs) was counted. The CFU count was repeated for each microtiter experimental plate.

Each microtiter plate included the appropriate controls, as well as a set of calibration wells.

The tested materials were examined 1 hour after polymerization and are hereinafter designated as "fresh samples." Similar experiments were performed in which, after polymerization, the tested materials were aged in 280 μL of phosphate-buffered saline (PBS) (Sigma Chemical) for 1 or 7 days before

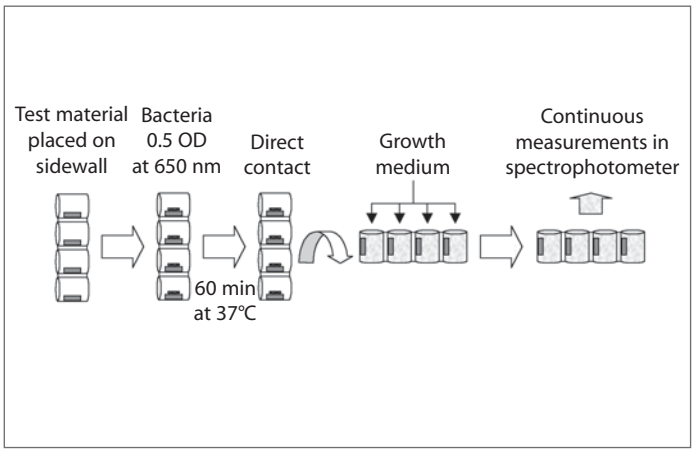

Fig 1 Schematic representation of the DCT experimental setup. The test is performed in a 96-well, flat-bottom microtiter plate. The plate is held vertically and the sidewalls of the wells are evenly coated with the test materials. A 10-μL bacterial suspension is placed on the test material. Evaporation of the suspension's liquid (1 hour at 37°C) ensured direct contact between bacteria and the test materials. The plate is then held horizontally and growth medium is added to each of the wells, followed by gentle mixing for 2 minutes. The plate is incubated at 37°C in the VersaMax microplate photospectrometer, where bacterial outgrowth is monitored. OD, optical density.

assaying. During the aging period, the PBS was renewed every 24 hours.

Statistical analysis

The values of the negative control wells were considered baseline and were subtracted from the respective experimental data, which were then plotted as growth curves. The curves for each well were analyzed, and a regression line was calculated on the ascending linear portion of the curve, expressed by the simple function $y = ax + b$. The formula of the linear portion provides 2 parameters: the slope, indicating growth rate; and the constant, correlating with the number of bacteria at time 0. Analysis of variance (ANOVA) and Tukey multiple comparison were applied to the 2 parameters.

RESULTS

Using the ADT, each of the tested cements was tested in sextuplicate. An inhibitory halo of 2.0 ± 0.3 mm and of 1.2 ± 0.2 mm was measured around the GC FujiCEM and Principle samples, respectively. No halos were observed around samples of the other cements.

The calibration experiments showed that bacterial outgrowth in microtiter wells could be monitored in a quantitative and reproducible manner (see Fig 1). Each point on the growth curve was the average of the optical densities measured in 3 wells at the same time. The gradual decrease in viable bacteria, due to serial dilutions at time 0, had virtually no effect on the bacterial growth rate or final density of bacteria at the stationary phase in the system.[16] DCT allows estimating the number of viable bacteria at the end of the direct contact incubation period using the calibration growth curves. The fifth- and the sixth-fold delusions represent 64 ± 5 and 12 ± 2 viable bacteria (CFU) per well, respectively. The DCT was performed on 8 specimens of each of the 4 materials tested. A regression line was preformed on the linear portion of the curve, which represents the logarithmic growth phase. The R^2 of all the growth curves ranged from 0.92 to 0.99. Two-way ANOVA showed a significant difference in bacterial growth rate, both on the different cements ($P < .001$) and at different tested time points ($P < .001$).

On fresh samples of Principle or GC FujiCEM, in experimental group A, no bacterial growth was observed. The optical density plotted for wells containing fresh samples of RelyX ARC and Variolink II showed a significantly different slope than that of the control ($P < .001$) (Fig 2, Table 1). After aging the materials for 1 day, bacterial growth was observed in all the wells containing the tested samples (group A). The plotted optical density in wells containing RelyX ARC, GC FujiCEM, or Principle differed from that of the control, only the latter being statistically significant ($P = .004$) (Fig 3, see Table 1). Upon aging the materials for 7 days, the plotted optical density did not differ from that of the control (see Table 1). In all tested plates, experimental group B wells demonstrated growth with no statistical difference from the control.

DISCUSSION

Caries is still the most common cause of FPD failure. The mean survival time of fixed partial dentures, due to caries, ranges from 8.3 to 16.0 years.[6–9] Although physically superior cements are important, longevity of the cemented restorations depends on the cement-dentin and cement-restoration interfaces, which provide a potential pathway for the penetration of cariogenic microorganisms. Therefore, the antibacterial properties of the cements play an important role in caries control adjacent to FPDs.

Using the DCT, the results of this study indicate a short-term antibacterial effect of some of the tested cements. This effect faded quickly: Upon aging the samples for 1 week, none of the cements retained an antibacterial effect. The group B wells indicated that cements in this study had only a bacteriostatic effect and not a bacteriocidic one. Recently, the antibacterial properties of the zinc phosphate and polycarboxylate cements were tested by DCT and ADT.[21] According to

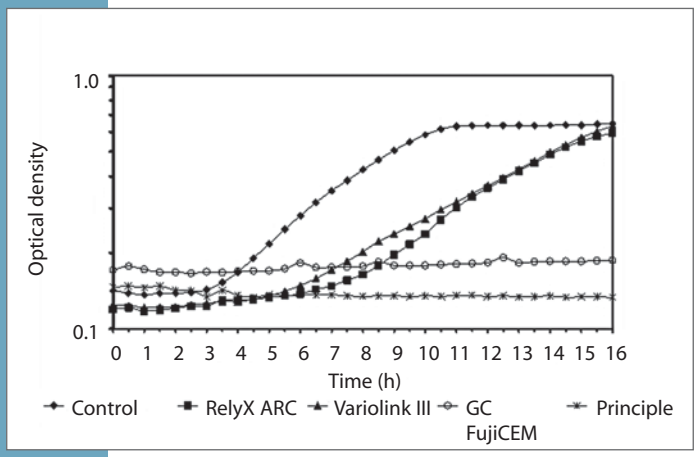

Fig 2 Bacterial outgrowth on the surface of fresh cement samples (1 hour after polymerization), measured by changes in optical density. Each point is the average of the optical densities measured in 8 wells at the same time.

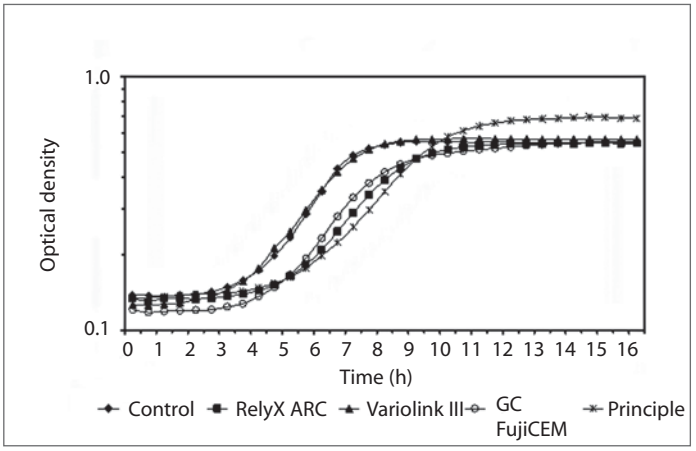

Fig 3 Bacterial outgrowth (measured by changes in optical density) on the surface of cement samples aged for 24 hours. Each point is the average of the optical densities measured in 8 wells at the same time.

Table 1 **Bacterial growth rate as reflected by the slope of the linear portion of the growth curve***

Time/material	Fresh material	24 hours	1 week
Control	0.0354 ± 0.0015	0.0552 ± 0.0034	0.0578 ± 0.0027
Variolink II	0.0183 ± 0.00607	0.0563 ± 0.0033	0.0448 ± 0.0126
RelyX ARC	0.0207 ± 0.0071	0.0448 ± 0.0079	0.0495 ± 0.0145
GC FujiCEM	0.0008 ± 0.00046	0.0467 ± 0.0104	0.0445 ± 0.0061
Principle	0.0003 ± 0.00025	0.0391 ± 0.0078	0.0548 ± 0.0033
One-way ANOVA	$P < .001$	$P = .004$	No significance

*Each value is the average of the slope of bacterial growth in 8 separate wells in the same microtiter plate. Vertical lines connect values that do not differ significantly (Tukey comparison).

the DCT, these cements maintained their antibacterial properties for at least 3 months. The ADT showed no halo around the samples, indicating low or no solubility of the antibacterial components in the surrounding aqueous milieu.

In the present study, the resin-modified glass-ionomer cement GC FujiCEM and the compomer cement Principle were the only cements exhibiting bacteriostatic activity according to the DCT and antibacterial activity according to the ADT. These findings may indicate that the antibacterial components in these cements are soluble in the aqueous media of the agar plate. Further indication to the solubility of GC FujiCEM components is demonstrated in the DCT (see Fig 2). Increased optical density values measured throughout the 16-hour experiment, identical to the negative control (not shown), indicate that this finding is not due to bacterial growth but to material solubility. Following polymerization, the probably short-term solubility of the latter cements (GC FujiCEM) should be clinically considered when using this class of materials.

Microleakage studies have suggested that resin cements are superior to compomer cements and to resin-modified glass-ionomer cements or traditional glass-ionomer cements in their sealing ability.[23,24]

The stability of the cement is an important factor in the prevention of microleakage and, subsequently, in the prevention of marginal caries. Glass-ionomer cement and its derivatives, resin-modified glass-ionomer cement and compomer cement, were considered to be advantageous in caries prevention because of their alleged ability to serve as a fluoride reservoir and therefore prevent

caries.[25–28] The current study may indicate that any antibacterial advantage is a short-term one, and it is likely associated with dissolution of a component of the material, which in turn may affect its stability.

It bears mention that both methods, the DCT and the ADT, are a departure from the clinical situation in terms of oral environment, margin location, surface area of cement exposed at restoration margins, and repeated occlusal load. Therefore, the results should be interpreted with caution. Furthermore, the observed antibacterial effect in this study was specific to *Streptococcus mutans*, the most common caries-related microorganism. Whether the antibacterial properties of the cements are effective against other clinically relevant bacteria, such as periopathogens, merits further investigation.

CONCLUSIONS

1. None of the tested cements in this study possesses long-term antibacterial properties.
2. Cements in this study had only a bacteriostatic effect and not a bacteriocidic one.
3. The antibacterial properties are likely associated with dissolution of a component of the material, which in turn may affect its stability.

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