Antibacterial Properties of Temporary Filling Materials

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Abstract
The purpose of this study was to investigate the antibacterial properties of temporary fillings. The direct contact test (DCT) was used to evaluate the antibacterial properties of Revoltek LC, Tempit, Systemp inlay, and IRM. These were tested in contact with Streptococcus mutans and Enterococcus faecalis. The materials were examined immediately after setting, 1, 7, 14, and 30 days after aging in phosphate buffered saline (PBS). Statistical analysis included two-way ANOVA, one-way ANOVA, and Tukey multiple comparison. Systemp inlay, Tempit, and IRM exhibited antibacterial properties when in contact with S. mutans for at least 7 days. Tempit and IRM sustained this ability for at least 14 days. When in contact with E. faecalis Tempit and IRM were antibacterial immediately after setting, IRM sustained this ability for at least 1 day. Our study suggests that the difference in temporary filling materials may influence which microorganism will be able to invade the root canal system. (J Endod 2006;32:214–217)

Key Words
Antibacterial, direct contact test, Enterococcus faecalis, Streptococcus mutans, temporary fillings

Temporary fillings are commonly used to seal endodontic access cavities between visits and after completion of endodontic therapy to prevent coronal microleakage (1). These materials can be divided into different groups according to their composition: reinforced zinc-oxide eugenol based materials, calcium sulfate-based materials, composite resin based materials, resin reinforced glass-ionomer materials, and traditional glass-ionomer materials.

The literature deals extensively with dye and bacterial microleakage of temporary fillings under different conditions (2–5). This microleakage was suggested as one of the causes for root canal treatment failures (6). Enterococcus faecalis is the most commonly recovered bacterial species from root canals after unsuccessful endodontic treatment (7–11). Love demonstrated in vitro invasion of E. faecalis into radicular dentin (11) This species is not susceptible to Ca(OH)₂ (12). It was suggested that the critical pH of 11 required to kill these bacteria is not reached in the dentin (13).

Streptococcus mutans is an inhabitant of the oral microflora and is the main bacteria associated with caries, and eventually the cause of pulp pathology (14). Antibacterial properties of restorative materials have been evaluated in vitro using various methodologies (15–18). Agar diffusion test (ADT) was the standard assay in most of these studies, despite its limitations (19). Weiss et al. introduced a 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 diffusivity of their components (20–25).

The purpose of this study was to investigate the antibacterial properties of four different temporary fillings, as these may decrease the risk of caries development and failure of endodontic therapy.

Materials and Methods

Tested Materials
Four commonly used temporary filling materials were tested. A composite resin based material: Revoltek LC (GC Corporation, Tokyo, Japan), calcium sulfate-based material: Tempit (Centrix, Shelton, CT), composite resin based material: Systemp inlay (Vivadent, Schaan, Liechtenstein) and a reinforced zinc-oxide eugenol based material: IRM (Dentsply International Inc., York, PA).

Test Microorganism and Growth Conditions
S. mutans 27351M, bacitracin resistant, was grown aerobically from frozen stock cultures in brain heart infusion (BHI) broth containing 0.5% bacitracin at 37°C. Clinically isolated E. faecalis, streptomycin resistant, was grown aerobically from frozen stock cultures in BHI broth containing 1.25% streptomycin at 37°C.

The DCT is based on turbidometric determination of bacterial growth in 96-well microtiter plate. The kinetics of the outgrowth in each well was recorded at 650 nm for 16 h every 30 min using a temperature-controlled spectrophotometer set at 37°C (VersaMax, Molecular Device Corp, Menlo Oaks Corp Center, Menlo Park, CA). Two sets of 8 wells for each tested material containing an equal volume of liquid medium were used, either in the presence (group A), or in the absence of the tested material (group B). No growth in group A wells and growth in the respective group B wells, indicates a bacteriostatic activity, while no growth in both groups indicates bactericidal activity. The experimental set-up of the DCT was described in detail by Weiss et al. (20–25).

The recorded data were plotted as semi-logarithmic growth curves. The linear portion of the curve, which correlates with bacterial growth rate, was transferred and
Results

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 five; each point on the curve is the average of three wells measured at the same time (not shown). The calibration growth curve allows estimation of the number of viable bacteria at the end of the incubation period.

DCT was performed on eight specimens of each of the materials tested. A regression line was performed on the linear segment of the curve in group A wells, which represents the logarithmic phase of growth. The $R^2$ of all growth curves ranged between 0.99 and 0.92. Two-way ANOVA, performed on all experiments, indicates a significant difference in bacterial growth rate (slope) between the two microorganisms in a combination of time and material ($p < 0.001$). For each microorganism the two-way ANOVA also indicates a significant difference in a combination of time and material ($p < 0.001$).

Testing *S. mutans* immediately after placement of the materials: Systemp inlay, Tempit, and IRM exhibited potent antibacterial properties, Tempit and IRM were bacteriocidic as there was no growth in group B wells. Revoltek LC was similar to the control and did not exhibit any antibacterial properties (Table 1).

After aging the materials for 1 day Systemp inlay, Tempit, and IRM kept their antibacterial properties, Tempit and IRM were still bacteriocidic, whereas Revoltek LC encouraged *S. mutans* growth (Table 1).

Revoltek LC was similar to the control after aging the materials for 7 days whereas Systemp inlay, Tempit, and IRM kept their antibacterial properties, Tempit and IRM were bacteriocidic (Table 1, Fig. 1).

After aging the materials for 14 days only Tempit and IRM were still bacteriocidic (Table 1). Aging the materials for 30 days proved to deny any antibacterial ability of all materials (Table 1).

When testing *E. faecalis* immediately after materials placement only Tempit and IRM exhibited antibacterial properties, Tempit was bacteriocidic whereas IRM was bacteriostatic. Systemp inlay and Revoltek LC were similar to the control and did not exhibit any antibacterial properties (Table 1, Fig. 2).

After aging the materials for 1 day only IRM kept its bacteriostatic property, whereas Systemp inlay, Tempit, and Revoltek LC showed no antibacterial phenomena (Table 1). None of the materials exhibited any antibacterial abilities after aging for 7 or 14 days (Table 1).

Discussion

Previous studies have suggested that the type of material used for temporary filling may result in microleakage (5–7, 26). Our study suggests that a difference in their antibacterial properties may play some role in determining which microorganism will colonize the dentine tubules or the root canal system and become the dominant pathogen (27).

The DCT is a method that provides information on bacterial viability and growth rate (20–25). It allows estimation of the number of viable bacteria at the end of the direct contact incubation period using the calibration growth curves. Changes in the slope of a growth curve, relative to the control, can be attributed solely to bacteria-material interaction.

**Table 1.** Bacterial growth rate: *Streptococcus mutans* and *Enterococcus faecalis* growth rate, as demonstrated by the slope of linear portion of the growth curve. Each number in the table is the average optical density (OD) ± standard deviation of the slope of bacterial growth in eight separate wells in the same microtiter plate. Vertical lines connect values that do not differ significantly (Tukey’s comparison).

<table>
<thead>
<tr>
<th>Time/Material</th>
<th>1 Day</th>
<th>7 Days</th>
<th>14 Days</th>
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<tr>
<td>Bacteria</td>
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<tr>
<td><em>S. mutans</em></td>
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<tr>
<td>Control</td>
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<td>0.0031</td>
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<tr>
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<tr>
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Revoltek LC a composite resin based material, did not exhibit any antibacterial activity regardless of bacteria or time. This material was found to encourage bacterial growth, although statistically significant only in contact with S. mutans 1 day after polymerization. The ability of bacteria to prosper on recently polymerized composite resin was previously observed (15, 23).

Systemp inlay a composite resin based material that contains triclosan, showed a statistically significant antibacterial activity when in contact with S. mutans for at least 7 days after polymerization. Systemp inlay in contact with E. faecalis did not exhibit any antibacterial activity. Triclosan is a chlorophenol widely used as an antibacterial chemical (28). The added triclosan may well be the explanation for the different results between Revoltek LC and Systemp inlay that are both composite resin based materials.

Tempit contains calcium sulfate and zinc oxide. Tempit in contact with S. mutans showed a statistically significant bacteriostatic activity up to 14 days after the material’s moisture activated setting, and when in contact with E. faecalis exhibited bacteriostatic activity only with fresh material. It is notable that, with both bacteria, some optical density was registered in the spectrophotometer when measuring Tempit as a fresh material. This is a result of the material’s solubility as shown by its similarity to the negative control when no bacteria were present.

IRM is a polymer reinforced zinc oxide-eugenol based material. IRM in contact with S. mutans showed a statistically significant bacteriostatic activity up to 14 days after setting, and when in contact with E. faecalis it exhibited bacteriostatic activity for at least 1 day.

Our study suggests that the antibacterial properties of temporary fillings may serve as a selective barrier that eventually determines the bacteria that consequently penetrate the root canal system. For example, IRM, had bacteriostatic influence on the growth of E. faecalis for 1 day after its placement but no effect after 7 days, whereas the same material had bacteriostatic effect on S. mutans for at least 14 days.

Under the circumstances tested, using Tempit and IRM as temporary fillings may result in an advantage for the growth of E. faecalis over S. mutans. These materials kept their bacteriostatic effect on S. mutans for 14 days, whereas Tempit was bacteriostatic on E. faecalis only as a fresh material, and IRM remained bacteriostatic for at least 24 h. Recently published data showed no difference in marginal leakage between IRM and Cavit, also a calcium phosphate based material (29). Microleakage of the temporary fillings materials after a short period of time has been demonstrated in previous works (4–6). Balto showed that IRM started to leak after 10 days, whereas Cavit, leaked after 14 days (30).

Sjögren et al. reported that following a negative culture before root filling healing occurred in 94% of cases, whereas only 68% healing occurred when the culture was positive, suggesting that healing is less likely to occur in the presence of bacteria at the time of obturation (31). The most commonly bacterial species recovered were E. faecalis (7). Although the most important function of temporary filling materials during and after endodontic treatment is their sealing ability and prevention of microleakage (6), the findings shown may suggest the importance of the antibacterial properties of temporary fillings as the interim material. Furthermore, despite intracanal dressing between appointments of endodontic therapy, dressing of the root canals with calcium hydroxide was challenged by some studies that reported a residual flora after its use (13, 32–34), therefore, a temporary filling material possessing good sealability and bacteriocidal properties may be advantageous in preventing bacterial invasion.

As all tested materials in this study lost their antibacterial properties over time, the clinical implication of this finding is the need for replacement of the temporary filling after a relatively short period, as previously suggested (6).

This in vitro experimental design requires the use of a small amount of examined material, and the aging of this material in PBS for different periods of time replacing the PBS every 48 h. In an in vivo condition the amount of material used is considerably larger. Further research is needed to establish whether the difference between the in vitro and in vivo conditions can accentuate the phenomenon reported in our study and to establish the effect of temporary filling materials on other bacterial species and its in vivo relevance.

Our study suggests that the difference in temporary filling materials may have some effect on the invasion of different microorganisms into the root canal system, thus suggesting an additional explanation for recovering E. faecalis from failed endodontic treatments.

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References


