Resistance against bacterial leakage of four luting agents used for cementation of complete cast crowns

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ABSTRACT: Purpose: To assess the sealing properties of four luting materials used for cementation of full cast crowns. Methods: 40 human premolars were prepared with a chamfer finish line. Stone dies were fabricated and copings were waxed, invested and cast in gold. Ten samples (n=10) were randomly assigned to four groups. In two groups, resin modified glass-ionomer cements were used, ACTIVA BioACTIVE-CEMENT/BASE/LINER and FujiCem2; the third group received the self-adhesive resin cement Embrace WetBond, while the fourth group served as control with a zinc phosphate cement. After cementation, excess cement was removed followed by bench-set for 10 minutes. All samples were stored in water at 37°C and subjected to thermal cycling (×2000 between 5 and 55°C). Subsequently the occlusal surface was reduced exposing the dentin. After sterilization the specimens were subjected to bacterial microleakage with E. faecalis in a dual chamber apparatus for a period of 60 days. Bacterial leakage was checked daily. Data were analyzed using the Kaplan-Meyer survival test. Significant pairwise differences were analyzed using the Log Rank test and the Fishers’ exact test at P< 0.05. Results: ACTIVA BioACTIVE-CEMENT/BASE/LINER, FujiCem2 and Embrace WetBond showed the lowest microleakage scores and differed statistically significantly (P< 0.05) from zinc phosphate cement. (Am J Dent 2014;27:51-55).

CLINICAL SIGNIFICANCE: The resin modified glass ionomer luting agents ACTIVA BioACTIVE-CEMENT/BASE/LINER and FujiCem2 and the self-adhesive resin cement Embrace Wet Bond exhibited a much better seal against E. faecalis when used for the cementation of indirect full cast restorations in comparison to a zinc phosphate cement. The clinical relevance should be viewed favorably as zinc phosphate cement has been the gold standard that has successfully been used for many years.

Introduction

Inadequate marginal fit of complete cast metal or ceramic crowns, porcelain fused to metal restorations and inlays/onslays is one of the most important shortcomings that can affect the wash-out of a luting agent resulting in bacterial penetration between restoration and dentin, thus increasing the risk of recurrent caries and pulp inflammation, which in turn may compromise the durability of a restoration. As demonstrated by Piwowarcycz et al, the choice of an appropriate cementing agent may be a determining factor concerning the degree of microleakage. Zinc phosphate cement has historically been the cement of choice for cementation of indirect cast restorations. The phosphoric acid and zinc oxide react ionically to form a low pH amorphous mass. However, the cement does not bond to dentin and due to its solubility, chemical dissolution occurs leading to higher microleakage scores than other cements. In order to overcome this problem, glass-ionomer cements, resin-modified glass ionomer cements and resin composite cements were developed in recent last decades. More recently, new improved self-adhesive resin-based or resin-modified glass-ionomer cements were introduced and are currently being used by the dental profession. Resin-based luting cements can penetrate into the dentin tubules and exposed collagen network and bond to dentin through micro-mechanical interlocking. Resin-modified glass-ionomer cements are hybrid formulas and composed of fluoroalumino silicate glasses, polyacrylic acid and resin composites and contain photo or chemical initiators and methacylate monomers. They bond to dentin through a combined ionic bond between polyacrylic acid and hydroxyapatite and a micro-mechanical interlocking with collagen and dentin tubules.

Previous studies have shown that resin-based and resin-modified glass-ionomer luting materials possess superior mechanical properties over conventional zinc phosphate cements, which may be a factor in sealing capacity as well as in resistance to displacement. In this respect, all of these classes of luting agents have been extensively investigated, but to date the self-sealing ability against bacterial leakage of this new improved generation of self-adhesive resin or resin-modified glass-ionomer luting materials has not been fully investigated.

Therefore, the present in vitro study compared the self-sealing property of two resin-modified glass ionomers and a contemporary self-adhesive resin-based luting agent with a conventional zinc phosphate cement in complete cast gold crowns by means of a bacterial microleakage test. The null hypothesis tested was that there is no significant difference with respect to marginal bacterial leakage among the tested materials.

Materials and Methods

Specimen selection and preparation - The present study received exemption from the Ethics Committee of the Argentine Dental Association (AO367-2013). Forty intact caries-free permanent human maxillary and mandibular premolars with fully developed roots, extracted for orthodontic reasons and showing comparable crown length and size, were used. All teeth were prepared by a single operator as described by Pameijer et al. Briefly, the teeth were prepared free-hand using a #4138 high-speed diamond chamfer bur using copious oil-free water-cooling. The occlusal surface was reduced perpendicular to the long axis, penetrating the dentin, while a medium chamfer finish line was circumferentially established in dentin and was located approximately 0.5 mm beyond the
Table 1. Description of the cementing agents tested in the study.

<table>
<thead>
<tr>
<th>Material</th>
<th>Material type</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTIVA BioACTIVE-CEMENT/BASE/LINER</td>
<td>Paste/paste, dual syringe Direct dispensing through a mixing tip</td>
<td>Paste A: Diurethane dimethacrylate and other methacrylate-based monomers and oligomers, polyacrylic acid/maleic acid copolymer, water, barium borosilicate glass, silica, reducing agents, photoinitiators and colorants. Paste B: Diurethane dimethacrylate and other methacrylate-based monomers and oligomers, aluminofluorosilicate ionomer glass, silica and oxidizing agents.</td>
</tr>
<tr>
<td>FujiCem2</td>
<td>Paste/paste pack cartridge Direct dispensing through automixing tips</td>
<td>Paste A: Alumino silicate glass, HEMA, UDMA Paste B: Polyacrylic acid, quartz, water</td>
</tr>
<tr>
<td>Embrace WetBond</td>
<td>Paste/paste, dual syringe Direct dispensing through a mixing tip</td>
<td>Paste A: Diurethane dimethacrylate and other methacrylate-based monomers and oligomers, water, barium borosilicate glass, silica, reducing agents, photoinitiators and colorants. Paste B: Diurethane dimethacrylate and other methacrylate-based monomers and oligomers, barium borosilicate glass, silica and oxidizing agents.</td>
</tr>
<tr>
<td>Zinc phosphate cement</td>
<td>Powder/liquid</td>
<td>Powder: Zinc oxide, magnesium oxide Liquid: Phosphoric acid</td>
</tr>
</tbody>
</table>

Abbreviations: HEMA: 2-hydroxyethylmethacrylate, UDMA: Urethane dimethacrylate.

cemento-enamel junction. All preparations had a height of ± 5 mm with a total angle of convergence of approximately 12° while all line angles were rounded. A new bur was used for every three preparations.

From each individual tooth, preliminary impressions were made in Putty Soft, Type I body vinyl polysiloxane (President®) using individual custom-made acrylic trays. Final impressions were made with light body vinyl polysiloxane type III (President®) and dies poured in type IV extra-hard stone (Densell Mix®) following the manufacturer’s instructions. The stone dies were trimmed and one coat of die relief (Renfert Pico-Fit®) was applied taking care to stay ± 1.5 mm short of the margins of the preparation. Subsequently wax patterns were fabricated to model copings ± 0.5 mm thick with flat occlusal surfaces. Using standard laboratory techniques, the wax patterns were invested in phosphate-bonded investment and cast in Type III gold. The castings were divested and the fit checked for accuracy under a stereomicroscope at ×20 magnification. The internal fitting surface was air abraded with 50 µm aluminum oxide. At all times, other than when they were worked on, the teeth were stored in a sterile phosphate buffer (SPB) solution. After checking for fit and retention each tooth and matching coping were numbered, individually bagged and autoclaved. After sterilization, 10 samples were randomly assigned to each of the four treatment groups.

Cementation  The luting agents used in this study were ACTIVA BioACTIVE-CEMENT/BASE/LINER (ABC) and FujiCem2 (FC2), two resin-modified glass ionomer cements; Embrace WetBond (EWB) a self-adhesive resin-based cement; and a zinc phosphate cement (ZPC) the latter used as a control (Table 1). Prior to cementation the teeth were thoroughly rinsed with a sterile oil-free air/water-spray and dried with filtered compressed air. Care was taken to avoid excessive drying. Cementation was performed under aseptic conditions by a single operator and strictly according to the manufacturer’s instructions.

The castings were filled with the mixed cement and immediately seated. All samples mixed with ABC, FC2 and EWB had an oxygen inhibition gel (DeOxi®) applied to the margins to prevent the formation of an oxygen-inhibited layer. They were then kept under finger pressure for 3 minutes. The gel was left in place for 4 minutes, and then excess cement was removed with a scaler. After bench setting for an additional 10 minutes, the marginal fit was checked by visual examination and a probe and the samples were stored for 24 hours in SPB at 37°C. They were subsequently subjected to 2,000 thermal cycles in water baths at 5°C and 55°C with a dwell time of 35 seconds and once more stored in SPB for 7 days at 37°C.

In preparation for the bacterial leakage test, the occlusal surfaces of the gold copings were reduced until the dentin was exposed using a fine high-speed diamond bur with light pressure and copious water-cooling. This was followed by sanding the occlusal surface on wet garnet paper of 400 and 600 grit® to remove gold flash that may have been caused by the diamond bur. Subsequently the entire root surface 1 mm below the margin of the copings was coated with two layers of nail polish and the samples were prepared for bacterial microleakage.

Bacterial leakage setup - For this experiment, a slight modification of the dual chamber test apparatus described by Imura et al12 was used. The tip of 1.5 ml Eppendorf plastic tubes (upper chamber) was cut, and the samples were pushed (crown first) through the opening until approximately one-half of the crown protruded through the end. The junction between the sample and the tube was then sealed with two layers of cyanoacrylate (Ciano®) and covered with sticky wax making sure the crown margin was located in the upper chamber. The tubes were placed into glass vials (lower chamber) containing 10 ml of sterile trypticase soy broth (TSB) in such a way that the occlusal dentin/cement interface was submerged in the broth of the lower chamber (Figure). The junction between the tube and the glass vial was sealed with two layers of cyanoacrylate and finally covered with sticky wax. The entire test apparatus was sterilized with ethylene oxide gas for 12 hours and then incubated at 37°C for 72 hours to verify sterility. If the TSB broth showed turbidity, the test set-up was discarded and replaced by a new one and the process repeated.

Bacterial leakage test - This phase of the study was carried out by a microbiologist in a microbiology laboratory under strict sterile conditions. The upper chamber was filled with 1 ml of TSB containing 24-hour growth of Enterococcus faecalis ATCC 29212 (108 colony-forming units/ml). The inoculated
apparatus was incubated for 60 days at 37°C. The upper chamber was re-inoculated every 5 days with fresh cultures of the microorganism. The TSB broth in the lower chamber was checked daily for turbidity, which when observed, was an indication that bacterial leakage had occurred along the crown/ cement or cement/dentin interface. Once turbidity was detected the day of the observation was recorded. Samples from both chambers were then incubated on blood-agar plates to check bacterial viability by morphological observation and Gram staining. The number of teeth demonstrating bacterial leakage and the days on which leakage occurred were recorded for each group.

**Statistical analysis** - The length of time until leakage was detected was compared among the tested cements using the Kaplan-Meier survival analysis. Significant pairwise differences were analyzed using the Log Rank test and Fishers’ exact test. The selected level of statistical significance was P< 0.05. The presence of *E. faecalis* in the lower chamber was also checked.

**Results**

Before the bacterial leakage test, two samples cemented with FC2, one with EWB and one with ZPC showed evidence of contamination. They were sterilized again according to the procedure described above. The results for the tested cements are shown in Table 2.

ABC revealed turbidity at 58 and 59 days (one sample each) whereas no leakage was observed in eight samples (80.0%). FC2 showed turbidity at 57 and 60 days (one sample each). In this group, eight samples (80.0%) did not show leakage. EWB did not show turbidity until 55 (one sample) and 58 days (two samples). In this group no leakage occurred in seven samples (70%). In the ZPC group turbidity occurred at 12, 14 and 18 days (one sample each), 21 and 22 days (one sample each), 25 days (three samples) and 42 days (one sample). No leakage was observed in one (10%) sample. The median survival time (absence of bacterial leakage) could not be estimated for ABC, FC2 and EWB since it was greater than 60 days, the time interval covered by the experiment, while it was 40 days (with a 95% confidence interval of 30.1 – 49.9) for the ZPC. No significant differences (P> 0.05) were detected among ABC, FC2 and EWB, while these three materials significantly differed (P< 0.05) from the ZPC. Bacteriological testing of the contents of the lower chamber with the samples demonstrated leakage and revealed viable *E. faecalis*.

**Discussion**

In the present study, an in vitro method was used to analyze the penetration of bacteria at the margins of cast gold crowns luted with ABC, FC2 and EWB. A ZPC was used as control because it has been used by the dental profession for many years as the luting material of choice for gold crowns and inlays and because its physicochemical characteristics are well known.\(^3,5\) Controversy exists in the literature whether or not in vitro and in vivo microleakage testing correlate and if results from the former experiments can be correlated with the clinical situation.\(^13\) In clinical practice other factors such as material biocompatibility, ease of use and specific requirements for each individual case (e.g. height of residual tooth structure, preparation angle and location of the preparation margins) may influence the choice of a cement.\(^13\)

*E. faecalis* was chosen because they exist in the normal oral flora in humans and are frequently found in mixed infections with other aerobes and facultative anaerobes.\(^14\) Some aspects of our protocol need to be considered. For evaluation, a qualitative approach (presence or absence of turbidity) during a 60-day period was used to detect the presence of bacteria in the lower chamber. Although this observation period was similar to what literature whether or not in vivo microleakage testing correlate and if results from the former experiments can be correlated with the clinical situation.\(^13\) In clinical practice other factors such as material biocompatibility, ease of use and specific requirements for each individual case (e.g. height of residual tooth structure, preparation angle and location of the preparation margins) may influence the choice of a cement.\(^13\)

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![Figure. Schematic set-up of the bacterial leakage system. UCB: Upper chamber containing TSB broth with *E. faecalis*; TP: Tooth preparation; E: Exit for bacteria; CR: Cast crown; LC Lower chamber containing TSB broth.](image)

**Table 2. Number of samples showing turbidity per 20-day interval.**

<table>
<thead>
<tr>
<th>Group/Material</th>
<th>Observation period (days)</th>
<th>N</th>
<th>1 – 20</th>
<th>21 – 40</th>
<th>41 – 60</th>
<th>No leakage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTIVA BioACTIVE-CEMENT/BASE/LINER</td>
<td>10 0 0 2 8 (80.0 %)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>8 (80.0 %)</td>
</tr>
<tr>
<td>FujiCem2</td>
<td>10 0 0 2 8 (80.0 %)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>8 (80.0 %)</td>
</tr>
<tr>
<td>Embrace WB</td>
<td>10 0 0 3 7 (70.0 %)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>7 (70.0 %)</td>
</tr>
<tr>
<td>Zinc phosphate</td>
<td>10 3 5 1 1 (10.0 %)</td>
<td>10</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>1 (10.0 %)</td>
</tr>
</tbody>
</table>
It should be noted that the objective of this study focused only on crown margins placed on dentin. As has previously been demonstrated, the degree of microleakage is greater on dentin than enamel, therefore a worst-case scenario was tested. Another untested variable in this study was the cement thickness between the restoration and dentin. As per protocol, die-relief spacer was used in order to decrease the seating discrepancies of the castings. It has been previously shown that the marginal seal is not negatively influenced by the cement thickness. This is, however, a variable that deserves further research. Occlusal load stress, which normally occurs under in vivo conditions, is another variable that was not tested in the current study and should be taken into consideration when marginal leakage of cemented crowns is tested. Prior to being subjected to bacterial microleakage, all samples were thermal cycled, which is a method to simulate the long-term stresses to which a restoration is exposed under clinical conditions. However, it has been reported that higher microleakage values were registered when thermocycling was followed by load-cycling, an issue that also needs to be investigated more extensively. Although we used a well-established protocol to simulate the oral environment, the real clinical scenario is too complex and difficult to reproduce by means of laboratory experiments. The set-up used in this study however, constitutes a viable basis for comparison among the tested materials.

The results demonstrated that ABC, FC2 and EWB had significantly lower leakage scores than the control ZPC, suggesting that they provided an acceptable marginal seal against bacteria for up to 60 days. These results are in agreement with Rossetti et al., with respect to the higher leakage values demonstrated by a zinc phosphate formulation. The results are also in agreement with a recent investigation reporting that resin-modified glass-ionomer and self-adhesive resin-based cements demonstrated a significant reduction in leakage scores.

ABC and FC2 are two recently introduced materials, thus limited information on their physicochemical characteristics is available in the literature. A previous investigation by Fabianelli et al. reported that FujiCem, a resin-modified glass-ionomer cement, exhibited good sealing properties and good adaptation with the dental substrate. According to the manufacturer, the chemical composition of FC2 is comparable to that of FujiCem, however, the latter has improved flexural and bond strength properties. It would be speculation to assume that the chemical similarity between FujiCem and FC2 automatically means that the physicochemical interaction with the tooth surface is similar; they may behave differently.

In this study, the self-adhesive resin cement EWB resulted in bacterial leakage values comparable to ABC and FC2. In this respect, the results tend to support those of Radovic et al., who suggested that the hydrophilicity of self-adhesive resin cements provides a satisfactory and improved adaptation to the tooth substrate with improved moisture tolerance, thus contributing to a significant reduction in microleakage. Therefore fewer microorganisms are available to penetrate the dentin tubules, which in turn implicates less pulpal irritation.

In addition to its effective self-sealing ability, the possible antimicrobial benefits of fluoride ion release from the self-adhesive resin cement as well as from both resin-modified glass-ionomer cements can be considered as a contributing factor of defense against bacteria, thus reducing bacterial leakage in vitro, which is consistent with the in vivo findings and support previous observations related to the chemical composition of luting materials as a factor of influence in their sealing performance.

The higher leakage scores observed for the ZPC are in agreement with the results of others who suggested that the low resistance of this cement to bacterial ingress is attributed to the lack of micromechanical and chemical bond to dentin and is a consequence of its high solubility. The results of the current study are also consistent with those of Philips et al. and a clinical study by White et al., but contradict those of Tung & Coleman who demonstrated no detectable molecular lipopolysaccharide and dextran diffusion beneath cast-gold crowns luted with a ZPC. The contradicting data from one laboratory to another may be explained on the basis of using different experimental conditions as well as variables such as tooth preparation, treatment of the dentin surfaces and application of the luting material. It should be emphasized, however, that as demonstrated in earlier studies, the high rate of solubility manifested by ZPCs does not necessarily have clinical implications and restorations can remain functional for many years.

Within the limitations of this study, the results revealed that cast crowns cemented with ABC, FC2 and EWB provided an acceptable marginal seal up to 60 days and showed statistically significantly lower bacterial leakage when compared to ZPC. Therefore, the null hypothesis was rejected. However, the stability and the sealing properties of the tested materials at the restoration/tooth interface need to be further investigated over longer time periods.

References


