ORIGINAL ARTICLE

Bacterial microleakage of temporary filling materials used for endodontic access cavity sealing

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KEYWORDS
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Abstract  Background/purpose: Providing a tight coronal seal is key for the success of endodontic treatment, therefore the study aimed to assess bacterial microleakage of materials used for short- and long-term temporization.

Materials and methods: One hundred and twenty-eight human upper-third molars were divided into six experimental groups (n = 20) and two control groups: negative (n = 4) and positive (n = 4). The standardized access cavities were prepared and filled with: (1) Cavit; (2) Fuji II LC; (3) Fuji IX; (4) Voco Clip; (5) AdheSE and Tetric EvoCeram; (6) Excite and Tetric EvoCeram. The crown of each tooth was sectioned to obtain 5.5-mm-high disks, which were assembled in a standard setup for bacterial microleakage studies using Streptococcus mutans. The monitoring lasted 90 days. Kaplan–Meier survival analysis was performed.

Results: The lowest amount of leaking samples was found in AdheSE and Tetric EvoCeram (31.3%), Cavit (33.3%), and Excite and Tetric EvoCeram groups (35.3%), followed by Fuji II LC (66.7%), Voco Clip (83.3%), and Fuji IX (88.2%) groups. According to the day of microleakage, materials could be classified in three groups with statistically significant differences (P < 0.05). In the first group were Cavit (70 days), AdheSE and Tetric EvoCeram (68 days), and Excite and Tetric EvoCeram (65 days), in the second group were Voco Clip (44 days) and Fuji II LC (43 days), and in the third group was Fuji IX (21 days).

Conclusion: None of the tested materials were able to completely prevent bacterial microleakage. Adhesively bonded composites and Cavit offer better sealing compared with glass ionomer.

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Introduction

Removal of microorganisms and their by-products, which are the main cause of periapical and periradicular disease, remains the primary goal of endodontic treatment. Temporary filling materials, which are used during and after endodontic treatment until the final restoration is placed, should provide a tight seal of the access cavity, thus preventing reinfection of the root canal system. A wide variety of temporary filling materials differing in their physical and chemical properties as well as adhesion and sealing properties are used in everyday practice. The most common materials for short-term temporization of the access cavity are zinc oxide eugenol (such as IRM, Dentsply Int., Milford, DE, USA) and zinc oxide/calcium sulfate (Cavit, 3M ESPE, St. Paul, MN, USA). Zinc oxide eugenol materials possess antimicrobial properties, making them more resistant to bacterial penetration, while the good sealing properties of zinc oxide/calcium sulfate materials can be explained by setting expansion and water sorption. For long-term temporization more durable materials, such as glass ionomer cements (GIC), which chemically bond to hard tooth structure or resin-based materials including composite resins and resin modified glass ionomer cements (RMGIC), are preferred. Unfortunately, resin-based materials including composite resins and RMGICs shrink (1.5–6%) during polymerization, resulting in the formation of gaps. To prevent gap formation and to establish retention and prevent leakage, most of these materials are bonded through the use of an adhesive system. Etch and rinse adhesive systems, with the application of 37% phosphoric acid, remove the smear layer and demineralize the surface layer of dentin. This exposes the network of collagen fibrils, which offers a predictable substrate for bonding. Hydrophilic monomers in the primer infiltrate the collagen network to form the hybrid layer. Most self-etching systems don’t remove the smear layer completely but rather include it into a hybrid layer. With the use of contemporary adhesive systems both approaches offer strong micromechanical and chemical bonds, which counteract the polymerization stresses of composite material during setting. Nonetheless, studies show that in certain areas of the cavity surface, shrinkage stresses may exceed adhesive bond strengths resulting in gap formation.

Several studies have used different methods to evaluate the sealing properties of temporary filling materials used for access cavity sealing. However, comparison of the results obtained with different methods is often difficult and unreliable. Even studies employing the same bacterial microleakage model have shown contradictory results. As well as this, newer materials are emerging on to the market every day. Therefore, there is a need for further studies evaluating the sealing potential of currently used temporary filling materials.

The aim of the study was to compare microleakage of temporary filling materials in standardized access cavities of molars using a two-compartment bacterial microleakage model using Streptococcus mutans as the microbial tracer.

Materials and methods

Specimen preparation

In our study 128 upper molar teeth were collected and stored in saline for up to 3 months. Republic of Slovenia, National Medical Ethics Committee, Ljubljana approved the use of human teeth. The teeth were ultrasonically cleaned and checked under an operative microscope (OPMI Pico, Carl Zeiss, Oberkochen, Germany) for the absence of cracks. Afterwards the coronal portion of each tooth was cut off and a standardized, Class-I endodontic access cavity 4 mm wide at the floor of the pulp chamber and 4.5 mm wide at the occlusal surface, parallel to the long axis of the tooth was prepared using a standardized, conical, diamond bur set in a paralometer. The entrances to the root canals were enlarged using ProTaper SX instruments and Gates Glidden burs Size II and III under copious irrigation with 0.9% saline solution. The pulp chamber was mechanically cleaned and the access cavity was irrigated for 2 minutes with 2 mL of 2.5% NaOCl followed by 4 minutes irrigation with 4 mL of 0.9% saline solution. The teeth were randomly assigned to six experimental groups (n = 20). Dental filling materials used for short- and long-term sealing of the endodontic access cavity were applied inside the standardized endodontic access cavity following the manufacturer’s instructions (Table 1). The manufacturers and chemical composition of materials used in this study are shown in Table 2.

The teeth were left in saline for 24 hours to enable complete polymerization. To obtain 5.5-mm-high cylinder shaped samples with the filling material in the middle, sectioning of the crowns with two cuts perpendicularly to the long axis was performed using a precision saw (Isomet 1000, Buehler, Lake Bluff, IL, USA; Figures 1A and 1B). The samples were covered by three layers of nail varnish up to 1 mm from the dentin/material interface. Positive controls (n = 4) with no filling material and negative controls (n = 4), where the whole surface of the sample was covered in nail varnish, were also included in the study.

Bacteriological leakage set-up

The samples were fixed in a two-chamber bacterial model described in earlier studies. One layer of sticky...
Table 1 Application mode of the materials used in this study.

| Material               | Application mode                                                                                                                                 |
|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| Group 1 Cavit          | Material was applied in bulk technique                                                                                                               |
| Group 2 Fuji II LC     | Dentin conditioner was actively applied on all cavity walls using a microbrush and rinsed off after 10 s. The cavity was carefully dried, not to overdry the dentin, using a cotton pellet. Afterwards Fuji II LC was applied in 2 mm increments; each individually cured for 20 s with a LED curing light (Bluephase; IvoClar-Vivadent, Schaan, Liechtenstein; intensity 1200 mW/cm²). The use of a coating agent was omitted so it would not penetrate into potential gaps. |
| Group 3 Fuji IX        | The access cavity was prepared in the same way as in Group Fuji II LC. Fuji IX was applied in a bulk technique and left to cure. The use of a coating agent was omitted so it would not penetrate into potential gaps. |
| Group 4 Voco Clip      | Material was applied in a bulk technique and cured for 40 s.                                                                                         |
| Group 5 Tetric EvoCeram & AdheSE | AdheSE Primer was actively applied to all cavity walls for 30 s using a microbrush, and thoroughly dried afterwards. AdheSE Bond was applied for 15 s using a microbrush, light drying and curing with a LED curing light for 15 s followed. Tetric EvoCeram was applied in 2-mm increments; each was individually cured for 40 s. |
| Group 6 Tetric EvoCeram & Excite | 37% phosphoric acid was applied to dentin walls for 15 s, rinsed off and lightly dried. An adhesive system Excite was thoroughly rubbed into dentin using a microbrush, lightly dried and cured for 20 s. Tetric EvoCeram was applied in 2-mm increments; each was individually cured for 40 s. |

Table 2 The manufacturers and chemical composition of the materials used in this study.

| Material               | Manufacturer                  | Composition (% weight)                                                                 |
|------------------------|-------------------------------|--------------------------------------------------------------------------------------|
| Cavit                  | 3M ESPE, St. Paul, MN, USA    | Zinc Oxide (30–50%) Calcium Sulfate (1–30%) Barium Sulfate (0–20%) Ethylene Bis (Oxyethylene) Diacetate (10–20%) Talc (0–20%) Zinc Sulfate (5–10%) Poly (Vinyl Acetate) (1–5%) |
| Fuji II LC             | GC EUROPE, Belgium Leuven    | (Fuloro) Alumino silicate glass (100%)                                               |
| Fuji IX                | GC EUROPE, Belgium Leuven    | Alumino silicate glass (95%)                                                          |
| Voco Clip              | Voco GmbH Cuxhaven, Germany  | Polyacrylic acid powder (5%)                                                          |
| Tetric EvoCeram        | Ivoclar-Vivadent, Schaan, Liechtenstein | 2-hydroxyethyl methacrylate (5–10%) nonhazardous additions |
| Tetric EvoCeram & AdheSE | Ivoclar-Vivadent, Schaan, Liechtenstein | Urethane dimethacrylate (2.5–< 10%) Bis-GMA (2.5–< 10%) ytterbium trifluoride (2.5–<10%) ethoxyxylated biphenol A dimethacrylate (2.5–<10%) nonhazardous additions |
| AdheSE                 | Ivoclar-Vivadent, Schaan, Liechtenstein | Primer Phosphonic acid acrylate (25–50%) Bis-acrylamide (10–<25%) Nonhazardous additions Bond Dimethacrylates Hydroxyethyl methacrylate Highly dispersed silicon dioxide Initiators and stabilizers Activator Solvent Initiators |
| Excite                 | Ivoclar-Vivadent, Schaan, Liechtenstein | SiO₂ (silicon dioxide) Initiators and stabilizers Hazardous components: <53% dimethacrylates <15% hydroxyethyl methacrylate <11% phosphonic acid acrylate <20% alcohol <1% potassium fluoride |
wax, two layers of cyanoacrylate glue and three layers of
nail varnish were used for fixation and isolation of the
chambers. The whole set-up was sterilized with ethylene
oxide. The top chamber was inoculated with an overnight
culture of S. mutans ATCC 25175 in tryptic soy broth (TSB:
approx. 10^8 colony forming units/mL), the bottom cham-
ber was filled with sterile tryptic soy broth (TSB). The
S. mutans broth culture was weekly replenished in the top
chamber, and the bottom chambers were monitored daily
for turbidity (Figure 2). The day of leakage was defined
as the day that turbidity occurred in the bottom chamber
for each specimen. The presence of S. mutans in
the bottom chamber of the samples which showed
turbidity was confirmed by identifying the bacterial
isolate by inoculation on blood and chocolate agar and
incubation for 48 hours at 25°C/14°C/n°C O2. The experiment
lasted 90 days.

Statistical analysis

In each group the proportion of leaked samples, as well as
the mean day of leakage and standard deviation, were
estimated using Kaplan–Meier survival analysis (P < 0.05)
and the differences among groups were tested using the
log-rank test.

Results

According to the day of leakage the materials could be
classified in three groups with statistically significant dif-
ferences between each group (P < 0.05; Table 3). The first
group of materials had the longest duration of the seal on
average and included Cavit (70 days), AdheSE and Tetric
(68 days), and Excite and Tetric (65 days). In the second
group were Voco Clip (44 days) and Fuji II LC (43 days),
while the last group included Fuji IX (21 days: Figure 3).

Discussion

The results of this study show that none of the tested
materials was able to completely prevent bacterial
leakage during the time of our experiment. The smallest
number of leaking samples was observed in Groups AdheSE
and Tetric, Excite and Tetric, and Cavit. Good sealing in
the first two groups can be explained by the use of either
etch and rinse or self-etch adhesive systems, which
reduced polymerization contraction and improved mar-
ginal integrity. The effective sealing of adhesively bonded
composite materials is in agreement with the findings of
other bacteriological studies, which showed that adhe-
sively bonded composite materials offered the best long-
term temporization when compared with a glass-ionomer
materials or IRM.10,18 However, Celik et al11 demonstrated
better sealing properties of glass-ionomer cement Ketac
Molar Easymix (3M ESPE, St. Paul, MN, USA) when
compared with a flowable resin composite material Filtek
Flow (3M ESPE, St. Paul, MN, USA), which is not in agree-
ment with the results of our study. Conflicting results may
be attributed to differences in the bacterial markers used,
the teeth studied, and the setup of the bacteriological
model.

Endodontic irrigants used during root canal therapy can
cause histological and morphological changes in dentin.
Studies have shown an adverse effect of high concentration
NaOCl on bond strengths of adhesive systems to dentin,20–22
either through damaging the collagen network or inhibiting
polymerization of the adhesive systems by releasing free
oxygen.23 The use of 2.5% NaOCl in our study might have
reduced the bond strength of both adhesive systems to such
an extent that in some of the samples debonding of the
composite materials owing to high polymerization stresses
in a geometrically unfavorable endodontic access cavity
might have occurred. This could help explain why one-third
of adhesively bonded composite restorations leaked in the
90 days of our study.

When comparing the adhesive systems used, there was
no statistical difference in the percent and time of leaking
samples between Groups AdheSE and Tetric and Excite and
Tetric, although the two adhesive systems applied differ in
their mechanism of action on the smear layer and adhesion
to dentin. A study by Fawzy et al24 analyzed the effect of
etch and rinse (Excite) and self-etch (AdheSE) adhesive
systems on dentin surface morphology and bond strength to
dentin with and without the use of 5.25% NaOCl. Pretreat-
ment of dentin with NaOCl followed by the self-etching
primer of AdheSE removed the smear layer and opened the
dentinal tubules to a similar extent as when only 37%
phosphoric acid was used. Interestingly, the bond strength of AdheSE to NaOCl pretreated dentin was highest, when compared with bond strengths of Excite to NaOCl pretreated dentin or both adhesive systems to nonirrigated dentin. Higher bond strengths of a self-etch adhesive system to NaOCl pretreated dentin compared with an etch and rinse adhesive system would probably account for fewer microgaps forming during polymerization and less microleakage at the dentin–composite interface, which was not the case in our study.

The good sealing ability of Cavit, owing to its hygroscopic nature and high setting expansion, has previously been reported in many studies. However, Cavit lacks mechanical properties, therefore it is not advisable to use it in thin layers or in complex endodontic access cavities. A RMGIC, namely Fuji II, showed less favorable sealing than adhesively bonded composite materials or Cavit. Even though conventional GIC and RMGIC have the ability to chemically bond to hard tooth structures, bond strengths are still lower than those of adhesively bonded composite materials. Also, studies show that RMGICs shrink with

| Material          | Leaked samples (%) | Time range (d) | Mean day of leakage (d) |
|-------------------|--------------------|----------------|-------------------------|
| Tetric & AdheSE   | 31.3               | 3–48           | 67.8 ± 35.1             |
| Cavit             | 33.3               | 4–79           | 69.5 ± 34.6             |
| Tetric & Excite   | 35.3               | 1–38           | 64.6 ± 36.3             |
| Fuji II LC        | 66.7               | 1–55           | 42.9 ± 37.4             |
| Voco Clip         | 83.3               | 5–86           | 44.2 ± 36.1             |
| Fuji IX           | 88.2               | 1–74           | 21.1 ± 33.8             |

In our study the material was applied in a Class I endodontic access cavity in thickness of 5.5 mm, with no mechanical loading being performed, which helps explain the low number of leaking samples obtained for Group Cavit.

A RMGIC, namely Fuji II, showed less favorable sealing than adhesively bonded composite materials or Cavit. Even though conventional GIC and RMGIC have the ability to chemically bond to hard tooth structures, bond strengths are still lower than those of adhesively bonded composite materials. Also, studies show that RMGICs shrink with

Figure 2 Checking for turbidity. (A) Nonleaked samples; (B) leaked sample’s turbidity in the bottom chamber.

Figure 3 Kaplan–Meier survival curve.
Bacterial microleakage of temporary filling materials

comparable or higher shrinkage stress values compared with adhesively bonded composite materials. The moisture content of the environment affects shrinkage values of RMGIC to a larger extent than it does composite materials. Lower bond strengths, higher shrinkage stress values and over-drying of RMGIC during the preparation process might have accounted for more microgaps forming at the dentin—material interface. Higher leakage values of RMGIC compared with adhesively bonded composite materials are in agreement with dye leakage studies of Gerdolle et al. and Khoroushi et al. A high percentage of leaking samples were anticipated for Voco Clip, a composite material used without an adhesive system and similar polymerization shrinkage values as Fuji II LC, due to the absence of micromechanical or chemical bonds to tooth structure. Although water absorption of resin-based materials and RMGIC over longer periods (4–8 weeks) increases the volume of these materials, this cannot fully compensate for microgaps forming during polymerization. This fact is corroborated by the results of several studies, which show comparable or lower sealing properties of nonbonded composite materials (TERM, Clip, Fermi) compared with Cavit. The latter findings are in agreement with the results of our study.

Surprisingly, Fuji IX, a conventional GIC, had the least resistance to bacterial leakage, even though this material chemically adheres to hard tooth structures and has good sealing properties. Several explanations for these findings exist. Firstly, conventional GIC are sensitive to water absorption or dehydration especially at the beginning of the setting reaction. Prolonged storage in water erodes the surface of the cement, with hydrolysis and dissolution of some of the components resulting in lower surface hardness and flexural strength. A coating agent was not used, therefore dilution of the GIC during the setting reaction of the material could have been expected. Secondly, the bond strength of conventional glass-ionomer cements to dentin is lower than that of RMGIC and adhesively bonded composite materials. This prime polymerization shrinkage stress, which is released during setting, contributes to the formation of gap microleakage pathways at the tooth—restorative material interface.

Within the limitations of this study it was concluded that adhesively bonded composites and Cavit offer better sealing of Class I endodontic access cavity when no mechanical loading is applied compared with GIC, RMGIC and composites without the use of an adhesive system. There was no difference in microleakage between Tetric EvoCeram bonded by a total-etch or a self-etch adhesive.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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