



ORIGINAL ARTICLE

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



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KEYWORDS

adhesive system;
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materials

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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cements, resin modified glass ionomer cements, and composites without the use of an adhesive system.

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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.¹ 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.² 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.³ 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.³ Unfortunately, resin-based materials including composite resins and RMGICs shrink (1.5–6%) during polymerization, resulting in the formation of gaps.^{4,5} 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 micro-mechanical and chemical bonds, which counteract the polymerization stresses of composite material during setting.⁶ Nonetheless, studies show that in certain areas of the cavity surface, shrinkage stresses may exceed adhesive bond strengths resulting in gap formation.^{7–9}

Several studies have used different methods to evaluate the sealing properties of temporary filling materials used for access cavity sealing,^{10–15} however, comparison of the results obtained with different methods is often difficult and unreliable.¹⁶ Even studies employing the same bacterial microleakage model have shown contradictory results.^{10,11,17–19} 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 paralelometer. 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.^{10,17,19} 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/cm ²). 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%) (Fuloro) Alumino silicate glass (100%)
Fuji II LC	GC EUROPE, Belgium Leuven	Alumino silicate glass (95%) Polyacrylic acid powder (5%)
Fuji IX	GC EUROPE, Belgium Leuven	2-hydroxyethyl methacrylate (5–10%) nonhazardous additions
Voco Clip	VOCO GmbH Cuxhaven, Germany	Urethane dimethacrylate (2.5–< 10%) Bis-GMA (2.5–< 10%) ytterbium trifluoride (2.5–<10%) ethoxylated bisphenol A dimethacrylate (2.5–<10%) nonhazardous additions
Tetric EvoCeram	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
AdheSE	Ivoclar-Vivadent, Schaan, Liechtenstein	Solvent Initiators
Excite	Ivoclar-Vivadent, Schaan, Liechtenstein	SiO ₂ (silicon dioxide) Initiators and stabilizers Hazardous components: <53% dimethacrylates <15% hydroxyethyl methacrylate <11% phosphonic acid acrylate <20% alcohol <1% potassium fluoride

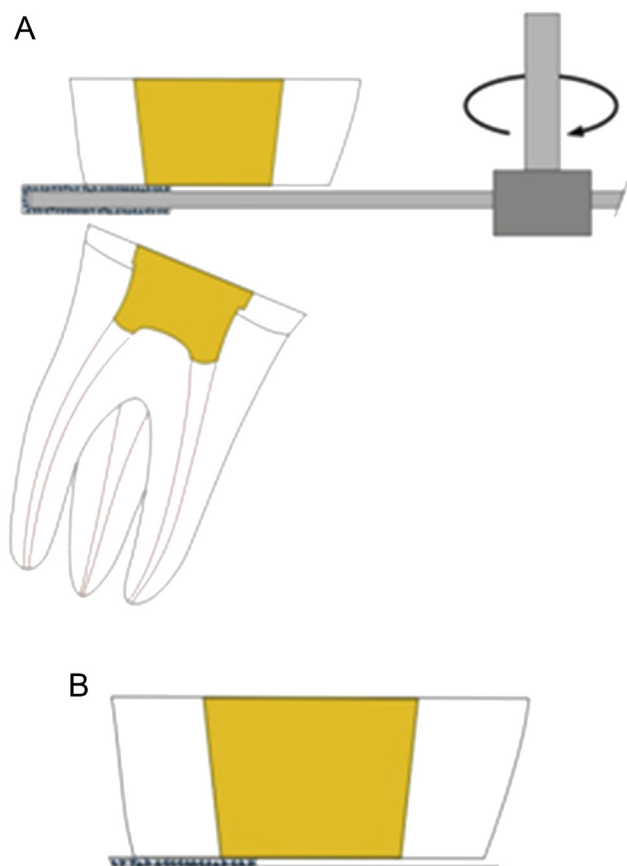


Figure 1 (A) Sectioning of the crowns filled with the dental filling materials used in this study; (B) obtaining 5.5-mm-high disks.

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 chamber 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 in CO₂. 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 differences 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 marginal integrity. The effective sealing of adhesively bonded composite materials is in agreement with the findings of other bacteriological studies, which showed that adhesively 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 agreement 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. Pretreatment 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%

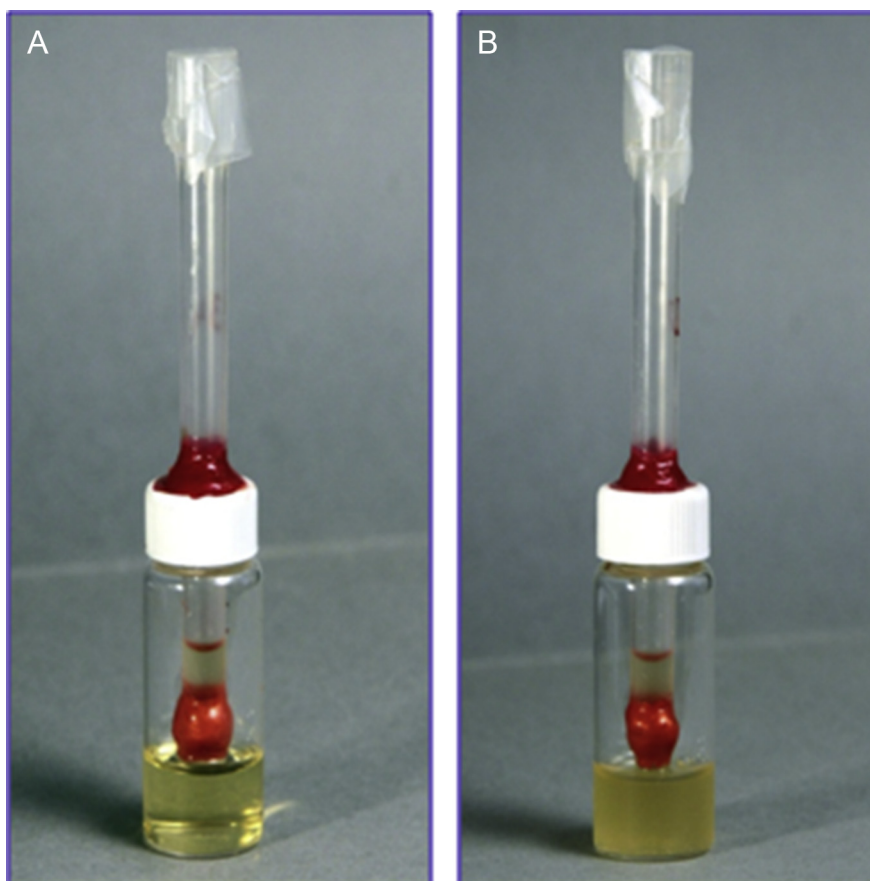


Figure 2 Checking for turbidity. (A) Nonleaked samples; (B) leaked sample's turbidity in the bottom chamber.

Table 3 Number of leaked samples and the estimated day of leakage.

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

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.^{12,14} However, Cavit lacks mechanical properties, therefore it is not advisable to use it in thin layers or in complex endodontic access cavities.²⁵

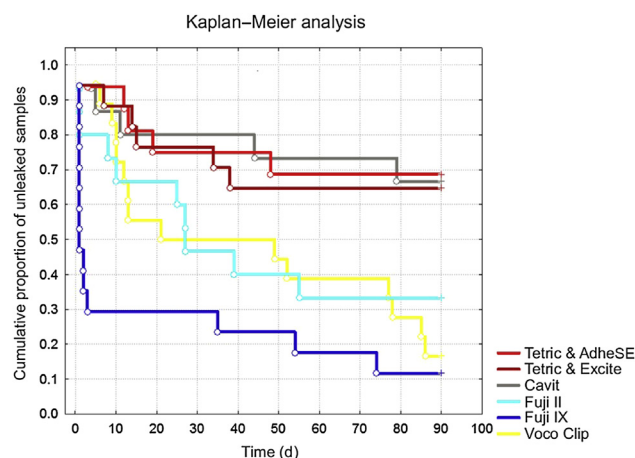


Figure 3 Kaplan–Meier survival curve.

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.²⁶ Also, studies show that RMGICs shrink with

comparable or higher shrinkage stress values compared with adhesively bonded composite materials.^{27,28} The moisture content of the environment affects shrinkage values of RMGIC to a larger extent than it does composite materials.^{29,30} 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 al²⁷ and Khoroushi et al.³¹

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.^{32–34} This fact is corroborated by the results of several studies, which show comparable or lower sealing properties of nonbonded composite materials (TERM, Clip, Fermit) compared with Cavit.^{14,35,36} 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.^{26,37} 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,^{26,38} thus debonding and microgap formation was more likely to occur. In contrast to our findings, Celik et al¹¹ and Barthel et al¹⁷ found that GIC offered the best sealing compared with other temporary filling materials, which can be explained by differences in the GIC material chosen for those studies.

The *in vitro* bacteriological model was used in this study and *S. mutans* was chosen as the test microorganism because it is a common oral pathogen and can survive in both aerobic and anaerobic conditions. As it is not a strict anaerobe, it can be used without the complexity of the oxygen-free experimental set-up that was used in our study. To prevent contamination of the lower chamber of the bacteriological setup, all manipulation was performed under laminar flow. The presence of *S. mutans* in the lower chamber was confirmed by morphological and biochemical characterization of the colonies on blood and chocolate agar. The bacteriological method was preferred over other leakage methods because examining resistance of materials to bacterial leakage is clinically most relevant. Furthermore, the model does not require destruction of the samples and enables examination of microleakage of the tested materials over a long period of time. For sample

preparation, an unfavorable geometry of the endodontic access cavity (Class I), with a high C-factor was selected, which would lead to increased polymerization stresses during setting of the tested materials and potentiate gap formation and microleakage pathways.³⁹

Although bacteriological methods give valuable information regarding microleakage, they have limitations. These are related to the hermetic joining of the two compartments, as well as identifying and quantifying microleakage pathways.⁴⁰ Therefore, future research should incorporate more sophisticated methods such as micro-computed tomography with its superior resolution and contrast imaging, which would enable 3D mapping of microleakage pathways (gaps) 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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