

# An Assessment of Microbial Coronal Leakage of Temporary Filling Materials in Endodontically Treated Teeth

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**This in vitro study evaluated the microbial leakage of Cavit, IRM, and Dyract when used as temporary filling materials after root canal treatment. The degree of coronal leakage was assessed by using a microbiological marker consisting of *Streptococcus faecalis* and *Candida albicans*. For each of the two organisms, a set of 15 maxillary premolars were prepared chemomechanically and obturated with thermoplasticized gutta-percha. A 3.5-mm thick layer of one of the three temporary filling materials was inserted in the access cavities of the teeth from each group (each group was compromised of five teeth). The control teeth (four positive and four negative) lacked any filling material over the gutta-percha, whereas the orifice and the apical foramen of the negative control were completely sealed with nail polish. Each tooth was placed in a well of a 24-well tissue culture plate and embedded in trypticase soy broth and 0.5% Bacto-agar. An organism suspension was inoculated in the access cavity, and microbial penetration was detected as an increase in turbidity of the broth. At the end of 30 days, the results showed that all positive control teeth leaked within 1 week, whereas those that served as negative control remained uncontaminated throughout the test period. With both organisms, IRM started to leak after 10 days, whereas Cavit and Dyract leaked after 2 weeks.**

A three-dimensional filling of the root canal system will prevent the penetration of microorganisms and toxins from the oral cavity via the root canal into the periradicular tissues. Coronal leakage of the root canal filling is considered to be an important cause of failure of root canal therapy (1). Weine (2) has indicated that improper restoration leads to loss of more endodontically treated teeth than actual failure of endodontic therapy. Good coronal

restoration resulted in significantly more absence of periradicular inflammation than good root canal treatment (3).

Studies of coronal leakage after completion of endodontic treatment have shown that the canal obturating techniques and materials do not provide a hermetic seal. Torabinejad et al. (4) showed that more than 50% of root canals were completely contaminated when the coronal surfaces of their fillings were exposed to *Staphylococcus epidermidis*. Similar observation was reported by Khayat et al. (5). In addition to bacterial infection, recently there has been a growing concern about yeast infections of the root canal. Microbiological investigations have shown that yeasts may be present in the microbial flora of apical periodontitis (6, 7). They may enter the pulp through dentinal tubules, deep carious lesions, fractures, or as contaminants from the oral microflora during the root-canal treatment (6, 8). Almost all isolated yeasts belong to genus *Candida*, and *Candida albicans* is the most often isolated species. So far there has been no study that compares the coronal leakage of the temporary restorative filling materials when *C. albicans* is used as a tracer.

The aim of this in vitro study was to evaluate the microbial leakage of Cavit, IRM, and Dyract when used as temporary filling materials after root canal treatment in premolars. *S. faecalis* and *C. albicans* were used as microbial tracers.

## MATERIALS AND METHODS

Thirty-eight extracted human maxillary premolars with intact crowns were used in this study. These teeth were extracted for orthodontic reason. The teeth were stored in 0.9% physiological saline and were kept moist at all times throughout the experiment. The periodontal ligament was removed from the root of the teeth by using a curette. To ensure uniformity in the root canal length, approximately 7 mm of the root length apical to CEJ was left intact, and the apical part was sectioned and removed by using a plain taper fissure bur in a high-speed handpiece under water spray. Access to the root canals was gained by using size #4 and #6 round burs in a high-speed handpiece under copious water spray. The access cavity was generally oval, 2.5 mm in width and 3.5 mm in length. Working lengths were designated as 1-mm short of the point at which a #20 k-file exited the apical foramen. The canals were instrumented to size 40k to obtain a standardized diameter of the apical end of the canals. Approximately 2 ml of 1% NaOCl

were used to flush the canal between each file size. Coronal flaring was accomplished with Gates Glidden burs, sizes 2 and 3. The specimens were steam autoclaved at 135°C for 20 min and after that all the procedures were performed under a laminar airflow hood using sterilized instruments. The canals were obturated with thermoplasticized gutta-percha, Obtura II (Obtura Corporation, Fenton, MO), using AH26 "silver free" as a sealer cement (De Tray Dentsply, Milford, DE). Gutta-percha was cold burnished with a ball burnisher at the apical end and vertically condensed coronally at the orifice opening of the canals. Excess root canal sealer was removed coronally with a sterile cotton pellet moistened in alcohol. The depth of the cavity was measured from the crest of the marginal ridge with a periodontal probe and was approximately 5.5 mm. Teeth were placed in an incubator at 100% humidity at 37°C for 72 h to allow setting of the sealer. All teeth were instrumented and obturated in the same manner by one operator and then randomly divided into 3 groups of 10 teeth each. A 3.5-mm thick layer of one of the three temporary filling materials was inserted in each group. Cavit (ESPE America, INC., Norristown, PA) was used in group 1 and IRM (L.D., Caulk Division, Milford, DE) in group 2. Both materials were placed incrementally in the access cavity with a plastic instrument, condensed with a plugger, and the excess material was removed with a sterile cotton pellet lightly dampened with sterile saline.

For the teeth in group 3, a thin layer of bonding agent was placed with the applicator on the dentinal wall of the prepared cavity and over the gutta-percha, cured with a visible light for 10 s, and then a 3.5-mm thick layer of Dyract (Dentsply-De Trey, Konstanz, Germany) was injected directly into the prepared cavity from the dispensing syringe and cured with the visible light activator for approximately 20 s. Group 4 consisted of eight teeth used as positive and negative controls (four teeth each).

The positive and the negative control teeth lacked any barrier over the gutta-percha. All surfaces of the negative control teeth, including the orifice and the apical foramen, were completely sealed with three layers of nail polish (Clarins, France). The teeth in the three experimental groups and the positive controls received three layers of nail polish leaving the area of canal's orifice and the apical foramen exposed. The thickness of the temporary filling materials was measured with a spring caliper (Hu-Friedy) with its one end placed at the proximal CEJ and the other end at the coronal level of the restoration.

The experimental and the control groups were divided into two equal subgroups, A and B. The teeth in subgroup A were inoculated with *S. faecalis*, whereas teeth in subgroup B were inoculated with *C. albicans*.

Before microbial inoculation, the filling materials in the access cavity and the surrounding tooth structure were disinfected with 30% hydrogen peroxide for 1 to 2 min and swabbed with 5% tincture iodine for approximately 2 min.

### Preparation of the Microorganisms

The cultures of *S. faecalis* and *C. albicans* were obtained from the Microbiology Laboratory, King Faisal Specialist Hospital and Research Center, Riyadh, Kingdom of Saudi Arabia. The cultures were maintained on blood agar Petriplates (nutrient agar containing 10% defibrinated sheep blood) until needed. The experiments were performed in plastic tissue culture clusters (Heerbrugg, Switzerland) containing 24 wells each with an inner diameter of 15 mm. Each tooth was placed in the middle of a well and embedded in a

TABLE 1. Means and standard deviations of starting day of microbial growth

Materials	Microorganisms	Mean	SD
IRM	<i>S. Faecalis</i>	10.00	2.24
IRM	<i>C. Albicans</i>	12.00	2.74
Cavit	<i>S. Faecalis</i>	16.40	1.34
Cavit	<i>C. Albicans</i>	15.20	1.64
Dyract	<i>S. Faecalis</i>	17.00	4.90
Dyract	<i>C. Albicans</i>	15.20	1.64
+ ve control	<i>S. Faecalis</i> & <i>C. Albicans</i>	6.00	0.00
- ve control	<i>S. Faecalis</i> & <i>C. Albicans</i>	30.00	0.00

bacteriological medium comprised of trypticase soy broth and 0.5% Bacto-agar (Detroit, MI) to cover the tooth up to CEJ (0.75 ml).

The microorganism suspension was made in phosphate buffered saline (PBS) containing 2% serum, and 50 ml of this suspension was inoculated in the cavity on top of the filling material. Every 48 h, fresh culture was added and approximately 100 ml of PBS was added to the bacteriological medium to keep it hydrated. The plates were incubated at 37°C, and the samples were monitored daily up to a maximum of 30 days. On a daily basis, the bottom of the tissue culture wells was checked visually for turbidity as compared with the negative control. If turbidity occurred, the day of leakage was recorded for each sample. After microbial growth was noticed, a sample from the medium was plated onto Columbia agar. The plate was checked by macroscopic morphological examination and Gram staining to assure that it contained the same type of organism as was placed in the prepared cavity.

Parametric two-way analysis of variance (ANOVA) was utilized to test the main factors (the organisms and the temporary filling materials) and the interaction between them. To check the significant difference between the materials, Tukey's multiple range test was utilized.

### RESULTS

The positive control teeth in groups A and B leaked within 1 week. Those that served as a negative control remained leakage-free at 30 days. A two-way ANOVA showed that there was no significant difference in terms of coronal leakage between the two organisms. Tukey's test showed a significant difference in the coronal leakage between IRM and Cavit ( $p = 0.002$ ) and between IRM and Dyract ( $p = 0.001$ ). With both organisms, IRM started to leak after 10 days, whereas Cavit and Dyract leaked after 2 weeks (Table 1).

### DISCUSSION

The importance of a well-sealed coronal restoration cannot be overemphasized. Many in vitro methods have been used to evaluate the sealing quality of endodontic filling materials. These methods are usually based on assessment of penetration of a tracer along the obturated root canal. The tracers most often used are dyes, radioisotopes, bacteria, or bacterial by-products (9). Isotopes and dye molecules, such as methylene blue, are much smaller than bacteria and most of bacterial by-products. Although isotopes and dyes may be good tools for comparing relative leakage, they do not simulate the types of microbial leakage that may occur clinically.

*S. faecalis* is a facultative anaerobic Gram-positive coccus. This bacterial species was chosen for use in this study because it is often involved in persistent endodontic infections (10) and is one of the most resistant species found in the oral cavity, having the ability to survive under unusual environmental stresses (11).

The model used in this study was sensitive, simple, and practical. Furthermore, the use of a culture on 0.5% Bacto-agar facilitates the penetration of the organism through the bacteriological medium.

A total thickness of 3.5 mm for all restorations was used in this study to comply with the recommendation of Webber et al. (12), who found that a 3.5-mm thickness of Cavit was the minimum thickness necessary to prevent total leakage of the dye molecule. The technique of placing these temporary filling materials into the access cavities might also have had some influence on the marginal leakage. In this study, all the materials were introduced into the access cavity by one operator to reduce the chances of a manipulative variable.

The results of this in vitro study indicate that Dyract and Cavit provide a better coronal seal than IRM. Dyract is a compomer material; such materials have gained acceptance among dentists due to their handling properties, esthetics, and fluoride release. In this study, Dyract was considered a semipermanent restoration until the tooth received the permanent one. It was chosen because it has bond strength significantly higher than that of all other fluoride-releasing materials (13).

The good sealing ability of Dyract is in agreement with the studies of Grobler et al. (13) and Uranga et al. (14), although their method of microleakage assessment was different than the one used in this study. The good sealing ability of Dyract may be attributed to the fact that it contains filled adhesives, which are believed to reduce polymerization contraction and improve marginal integrity (15). The use of a bonding agent before placing Dyract could be another reason for the better sealing of this material.

Khayat et al. (5) determined that in < 30 days bacteria present in natural human saliva will penetrate through an entire root canal system obturated by a lateral or vertical condensation techniques. Similar observation was made in this study when selected microorganisms were used.

The good sealing ability of Cavit in endodontic access preparations has been previously reported (16). The better sealing property of Cavit compared with IRM may be attributed to its relatively higher linear expansion during setting (17). Another reason might be that the material is premixed and it reduces the inconsistencies related to chairside mixing. Numerous investigations on microleakage have obtained varying results using Cavit. According to Lim (18), the contradictory reports on the microleakage of Cavit may be due to the differences related to the duration and methods of evaluating microleakage in the different studies.

The poor sealing ability of IRM may be linked to the fact that powder and liquid have to be mixed together to produce the paste to be inserted. This mixing is the cause of reduced homogeneity (19). In this study and in other studies on the performance of IRM with regard to bacteria (19, 20), the bacterial cells infiltrate along

the interface, despite the antibacterial effect of the eugenol in the cement.

Certainly this in vitro method of microleakage measurement cannot duplicate the environment that exists in vivo. However, the results from this study provide information that could aid the clinician in the selection of the best material to extend the leakage-free time period before the final restorative treatment is initiated.

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## References

1. Saunders WP, Saunders EM. Coronal leakage as a cause of failure in root canal therapy: a review. *Endod Dent Traumatol* 1994;10:105-8.
2. Weine FS. *Endodontic therapy*. 4th ed. St. Louis: CV Mosby, 1989:4.
3. Ray H, Trope M. Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration. *Int Endod J* 1995;28:12-8.
4. Torabinejad M, Ung B, Kettering JD. In vitro bacterial penetration of coronally unsealed endodontically treated teeth. *J Endodon* 1990;16:566-9.
5. Khayat A, Lee SJ, Torabinejad M. Human saliva penetration of coronally unsealed obturated root canals. *J Endodon* 1993;19:458-61.
6. Waltimo TMT, Siren EK, Torkko HLK, Olsen I, Haapasalo MPP. Fungi in therapy-resistant apical periodontitis. *Int Endod J* 1997;30:96-101.
7. Molander A, Reti C, Dahlen G, Kvist T. Microbiological status of root filled teeth with apical periodontitis. *Int Endod J* 1998;31:1-7.
8. Sen BH, Safavi KE, Spangberg LS. Growth patterns of *Candida albicans* in relation to radicular dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1997;84:68-73.
9. Al-Ajam A, McGregor A. Comparison of the sealing capabilities of Ketac silver and extra high copper alloy amalgam when used as retrograde root canal filling. *J Endodon* 1993;19:35-6.
10. Siren EK, Haapasalo MPP, Ranta K, Salmi P, Kerosuo ENJ. Microbiological findings and clinical treatment procedures in endodontic cases selected for microbiological investigation. *Int Endod J* 1997;30:91-5.
11. Jett BD, Huycke MM, Gilmore MS. Virulence of enterococci. *Clin Microbiol Rev* 1994;7:462-78.
12. Webber RT, del Rio CE, Brady JM, Segall RO. Sealing quality of a temporary filling material. *Oral Surg Oral Med Oral Pathol* 1978;46:123-30.
13. Grobler S, Rossouw R, Van Wyk Kotze T. In vitro, relative microleakage of five restorative systems. *J Dent Res* 1999;49:47-52.
14. Uranga A, Blum J, Esber S, Parahy E, Prado C. A comparative study of four coronal obturation materials in endodontic treatment. *J Endodon* 1999;25:178-80.
15. Kemp-Scholte CM, Davidson CL. Marginal integrity related to bond strength and strain capacity of composite resin restorative systems. *J Prosthet Dent* 1990;64:658-64.
16. Pisano DM, DiFiore PM, McClanahan SB, Lautenschlager EP, Duncan JL. Intraorifice sealing of gutta-percha obturated root canals to prevent coronal microleakage. *J Endodon* 1998;10:659-62.
17. Wideman FH, Eames WB, Serene TP. The physical and biologic properties of Cavit. *J Am Dent Assoc* 1971;82:378-82.
18. Lim KC. Microleakage of intermediate restorative materials. *J Endodon* 1990;16:116-8.
19. Deveaux E, Hildebert P, Neut CH, Boniface B, Romond CH. Bacterial microleakage of Cavit, IRM, and TERM. *Oral Surg Oral Med Oral Pathol* 1992;74:634-43.
20. Blaney TD, Peters DD, Setterstrom J, Bernier WE. Marginal sealing quality of IRM and Cavit as assessed by microbial penetration. *J Endodon* 1981;7:453-7.