

Microbial microleakage in temporary restorative materials after post space preparation

Infiltração microbiana em materiais restauradores temporários após preparo para retentores intrarradiculares

Abstract

Purpose: This study investigated the microbial microleakage through temporary restorative materials (Coltosol, IRM, Vidrion R) after post space preparation.

Methods: Forty-two maxillary anterior human teeth were prepared and obturated with gutta-percha and Sealapex using the lateral condensation technique, with 4mm of apical obturation remaining. Calcium hydroxide paste was used to fill the post space preparation, and 4mm of the coronal portion was restored with the temporary filling materials. Six specimens were totally sealed (negative control) and six specimens were not filled (positive control). An *in vitro* microbial leakage test (MLT) with a split chamber (upper/lower chambers) was used. Microbial microleakage was observed daily for 90 days. Data were analyzed by Kruskal-Wallis and Mann-Whitney tests.

Results: Coltosol, IRM, and Vidrion R allowed microbial microleakage after 19 to 89 days.

Conclusion: All temporary fillings and intracanal dressings did not prevent the penetration of microorganisms to the root apex.

Key words: Coronal leakage; bacterial leakage; temporary restorations; post; calcium hydroxide

Resumo

Objetivo: Este estudo investigou a infiltração microbiana em materiais restauradores provisórios (Coltosol, IRM, Vidrion R) após preparo para retentores intrarradiculares.

Metodologia: Quarenta e dois dentes humanos anteriores superiores foram preparados e obturados com guta-percha e Sealapex utilizando a técnica da condensação lateral, mantendo 4mm de remanescente apical de obturação. Foi usada pasta de hidróxido de cálcio para preencher o espaço criado para os pinos, deixando um espaço de 4mm na cervical a ser preenchido pelos materiais testados. Seis espécimes foram totalmente impermeabilizados (controle negativo) e seis espécimes não foram obturados (controle positivo). Um teste de infiltração microbiana (MLT) com uma câmara dividida em duas partes foi usado neste ensaio. A infiltração microbiana foi verificada diariamente durante 90 dias. Os dados obtidos foram analisados pelos testes de Kruskal-Wallis e de Mann-Whitney.

Resultados: Coltosol, IRM e Vidrion R permitiram infiltração microbiana após 19 a 89 dias.

Conclusão: O material restaurador temporário e a medicação intracanal não preveniram a penetração de microrganismos até o ápice radicular.

Palavras-chave: Infiltração coronária; infiltração bacteriana; restaurações temporárias; retentores; hidróxido de cálcio

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Received: November 26, 2007
Accepted: February 22, 2008

Introduction

Coronal and apical microbial microleakage along the coronal restoration and root canal filling has been strongly associated with endodontic failure. Rupture of coronal sealing and saliva microleakage may damage the apical sealing and compromise periapical health. These facts show the importance to guarantee adequate endodontic infection control by perfect coronal-root sealing.

Post space preparation, root canal filling, post cementation, and temporary coronal restorations may predispose the occurrence of microbial contamination and apical periodontitis. Endodontic treatment presents unfavourable prognosis when there is inadequate root canal obturation (1-5). Ray and Trope (3) observed in 1,010 radiographs of endodontically treated teeth that the technical quality of the coronal restoration was significantly more important than the technical quality of the endodontic treatment for apical periodontal health. On the other hand, the technical quality of the endodontic treatment has been shown to be more important than the technical quality of the coronal restoration (2,5). Kirkevang et al. (2) analyzed the radiographs of 773 root-filled teeth to examine the quality of endodontic treatment and coronal restorations as well as its association with periodontal status, and reported a prevalence of 52.3% of apical periodontitis. Inadequate root canal filling and coronal restoration were associated with increased incidence of apical periodontitis.

Previous studies compared the sealing ability of several temporary restorative materials using different methods (6-11). Teeth with post space preparation require caution with possible recontamination coronally (12-19), which can jeopardize the endodontic treatment success. Additionally, the influence of coronal microleakage on microbial contamination has been investigated (20-29). Clinical decision making should take into consideration the sealing performance of temporary restorative materials after post space preparation.

The objective of this study was to evaluate the sealing ability of temporary restorative materials after post space preparation in teeth with apical root filling remaining.

Methods

Test organisms

This experiment used a mixture of five microorganisms – four reference bacterial strains and one yeast strain, obtained from the American Type Culture Collection. Facultative bacteria included were *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), and *Bacillus subtilis* (ATCC 6633). The yeast used in this study was *Candida albicans* (ATCC 10231).

The microorganisms were inoculated in 7mL of Brain Heart Infusion (BHI; Difco Laboratories, Detroit, MI, USA) broth and incubated at 37°C for 24h. The experimental suspensions were prepared by cultivation of the biological

indicators on the surface of Brain Heart Infusion Agar (BHIA; Difco Laboratories, Detroit, MI, USA), following standardized incubation conditions; microbial cells were resuspended in saline to give a final concentration of about 3×10^8 cells/mL, adjusted to n° 1 MacFarland turbidity standard. One milliliter of each of these pure suspensions was used to obtain a mixture of the test microorganisms.

Microbial Leakage Test

Forty-two extracted human maxillary anterior teeth obtained from the Brazilian Dentistry Research and Learning Center (CEPOBRAS) were selected. Radiographs of each root were taken to verify the following inclusion criteria: single canal, absence of internal or external resorption or calcification, and complete apex. The teeth were stored in 0.2% thymol solution (Fitofarma, Goiânia, GO, Brazil), immersed in 5% sodium hypochlorite (NaOCl; Fitofarma, Lt. 20442, Goiânia, GO, Brazil) for 30min to remove any organic tissues. The crowns were removed and tooth length was standardized to 16mm (from root apex to coronal border).

After initial radiographs, standardized access cavities were prepared, and the cervical third of the canals was enlarged with ISO #70 to ISO #110 Gates-Glidden drills (Dentsply/Maillefer, Ballaigues, Switzerland). After this step, ISO #2 Largo drills (Maillefer, Switzerland) were used to prepare the intraradicular post space standardized to 11mm. The root canals were prepared up to ISO #50 K-File (Dentsply/Maillefer) at 1mm from the apex. During instrumentation, the root canals were irrigated with 3mL of 1% NaOCl (Fitofarma). After root canal preparation, an ISO 15 K-File (Dentsply/Maillefer) was passed through the apex to ensure patency. Root canals were dried and filled with 17% EDTA (pH 7.2) (Biodinâmica, Ibioporã, PR, Brazil) for 3min to remove the smear layer.

Teeth were obturated with gutta-percha and Sealapex (Kerr Sybron, Romulus, MI, USA) using the lateral condensation technique. The root canal filling material was removed with Paiva heated plugger # 2, leaving 4mm of apical root filling. Intracanal dressing (CHP) filled the post space, leaving 4mm of cervical space to be filled with the tested temporary restorative materials.

The teeth were randomly assigned to three experimental and two control groups, according to the temporary restorative materials, as follows: A (n=10), Coltisol (Vigodent, Rio de Janeiro, RJ, Brazil); B (n=10), Intermediate Restorative Material (IRM, Dentsply, Petrópolis, RJ, Brazil); C (n=10), Vidrion R (SS White, Rio de Janeiro, RJ, Brazil); D (n=6), Positive Control, no restorative material; E (n=6), Negative Control, complete sealing with nail varnish and Coltisol restoration. Half of the experimental specimens (15) had the post space portion filled with calcium hydroxide paste (CHP), corresponding to a toothpaste-like texture (calcium hydroxide P.A., Quimis, Mallinkrodt Inc., St. Louis, MO, USA) plus saline. The tested restorative materials were prepared according to each manufacturer's instructions. The teeth were wrapped in wet gauze and placed in an incubator

(Bacteriological Incubator, Odontobrás Ind. Com. Equip. Med. Odont. Ltda, Ribeirão Preto, SP, Brazil) at 37°C for 24h to allow complete setting of the filling materials.

A split chamber (upper and lower chambers) was used in the experimental model. The upper chamber had a microbial suspension with the biological markers, while the lower chamber contained the culture medium. Microbial mixture only would reach the lower chamber by leaking through the apical filling.

The coronal portion of the root canal was fixed to the end of a 1.5mL polypropylene Eppendorf tube (Cral, São Paulo, SP, Brazil) using cyanoacrylate adhesive (Super Bonder, Itapevi, SP, Brazil) and epoxy resin (Durepoxi, São Paulo, SP, Brazil). The tooth-tube interfaces were coated with two layers of nail varnish (Max Factor, Cosmetics and Fragrances, Los Angeles, CA, USA), except for 3mm of the apical root. The teeth of the negative control group were completely coated with two layers of nail varnish including the apical portion. The specimens (teeth coupled to the polypropylene tubes) were sterilized in 5% NaOCl for 30min and rinsed with sterile water for 30min.

The polypropylene tubes were attached to a rubber cover that was placed into a 10mL sterile glass flask containing the culture medium. The flasks were filled with 8mL BHI broth (Difco, Detroit, MI, USA), and 3 mm of the root apex was immersed in the broth. The specimens were placed into the culture medium (BHI) and the testing apparatus were incubated at 37°C for 24h. No growth was observed after this period.

The whole apparatus was incubated at 37°C. Fresh overnight cultures of organisms were added to the tubes at 7-day intervals (Fig. 1). Microbial leakage was assessed daily during 90 days, considering the turbidity of the culture medium as an indicator of microbial contamination. Positive BHI tubes were selected, and inocula were spread on BHI agar surface under identical incubation conditions. Gram stains of the BHI growth and from colonies growing on BHI agar were carried out.

Data were analyzed by Kruskal-Wallis test for significant differences among the filling materials and by Mann-Whitney test for the variation within groups (influence of CHP use). A significance level of 0.05 was adopted.

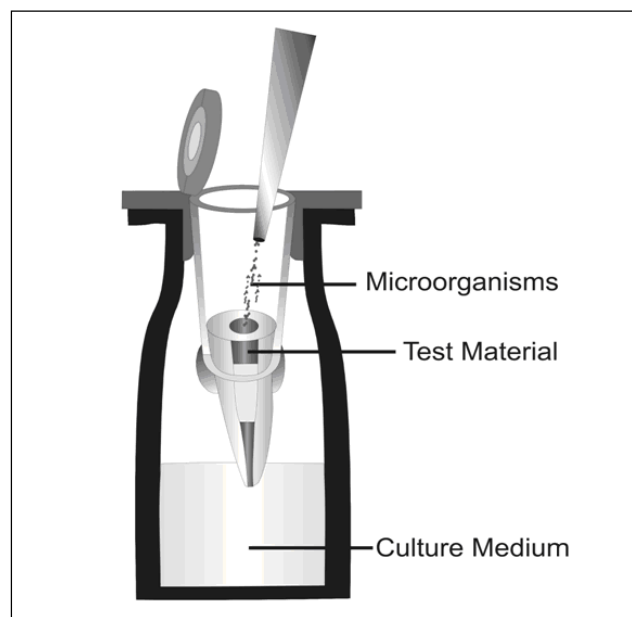


Fig. 1. Diagram of the microbial leakage test.

Results

The time period (days) when microbial microleakage occurred and the comparison of the test materials are displayed in Table 1. The results showed that Coltosol, IRM, and Vidrion R allowed microbial microleakage after 19 to 89 days. The presence of CHP did not influence the penetration of microorganisms through the 4mm apical root canal filling remaining. All positive control teeth presented microbial leakage, and none of the negative control showed leakage.

Table 1. Minimum and maximum periods (days) when microbial microleakage occurred and mean rank comparison among the materials tested.

Materials	n	Minimum (days)	Maximum (days)	Mean rank	P*
Coltosol without Ca(OH) ₂	5	60	65	17.80	0.167(a)
Coltosol with Ca(OH) ₂	5	68	82	19.00	
IRM without Ca(OH) ₂	5	43	83	9.00	
IRM with Ca(OH) ₂	5	70	71	19.20	
Vidrion R without Ca(OH) ₂	5	19	89	10.00	
Vidrion R with Ca(OH) ₂	5	36	71	17.60	
Coltosol	10	60	82	18.60	0.362(a)
IRM	10	43	83	14.10	
Vidrion R	10	19	89	13.80	
Without Ca(OH) ₂	15	19	89	15.67	0.914(b)
With Ca(OH) ₂	15	36	83	15.33	

* Kruskal-Wallis test (a), Mann-Whitney test (b).

Discussion

The current concept of a complete endodontic treatment involves perfect and definite coronal sealing. Some clinical conditions are necessary to prepare the post space, such as maintenance of a small apical root canal filling remaining and the use of temporary restorative materials. In these cases, these teeth present risk for microbial microleakage.

It is important to consider that after losing coronal sealing or dental fracture, microbial microleakage often occurs (14,22,27). This study analyzed the penetration of a poly-microbial marker through three temporary restorative materials (Coltosol, IRM, and Vidrion R) after removing the gutta-percha filling for post space preparation leaving 4mm of apical root filling remaining. The influence of CHP dressing in the post space portion also was studied. The results showed that all tested restorative materials did not prevent microbial microleakage over a 90-day period. The presence of CHP in the post space did not interfere on sealing ability.

The experimental protocol used was modified from previous studies (13-15,25,28). The extrapolation of those results to *in vivo* conditions is limited due to methodological details. The molecular size of most dye particles is smaller than that of bacteria, and, in addition, it is not viable to reproduce the interactions between microbial and non-microbial tracers and also between *in vitro* and *in vivo* tests (23). Therefore, MLT should be used (29). A polymicrobial culture (*S. aureus*, *E. faecalis*, *P. aeruginosa*, *Bacillus subtilis*, and *C. albicans*) was selected as biological indicators because of different structural aspects (Gram-positive coccus, Gram-negative rods, Gram-positive rods, yeast) (6,24,25).

Although in clinical situations the number of contaminating microorganisms has been shown to be relevant, the purpose of this study was to verify the sealing ability of provisional restoration materials in cases with intracanal dressing for post space and short apical root filling remaining. Thus, the quantity of microbial cells responsible for turbidity was not analyzed. The main focus was to evaluate the sealing potential of temporary restorations in teeth with post preparation.

During the period of microbial contamination in the present assay (90 days), the turbidity of the culture medium was used as an indicator of microbial microleakage (qualitative analysis). Chailertvanitkul et al. (20) reported that microleakage is positive only when there is turbidity of the culture medium, indicating complete penetration of microorganisms through the restoration and root canal filling. In addition, they stated that the number of microorganisms required to cause apical periodontitis is unknown.

In the present study, the post space was prepared during root canal instrumentation, and the root canal filling material was removed using a heated plugger immediately after canal filling (12,21). Haddix et al. (21) compared the effect of

post preparation techniques on apical sealing and suggested that heated pluggers should be used to remove gutta-percha. On the other hand, Abramovitz et al. (12) showed that the immediate post space preparation with hot pluggers did not differ from delayed preparation with drills in relation to quality of apical root canal sealing.

In the current study, Coltosol, IRM, and Vidrion R associated with 4mm of apical root filling remaining, with or without CHP, did not prevent microbial microleakage after 19 to 89 days. Comparison with previous findings should be conservative due to different methods and materials used. For example, Deveaux et al. (9) evaluated bacterial leakage in Cavit, IRM, TERM, and Fermit materials and reported that Cavit had good sealing properties for up to 21 days in a single endodontic access. In other study, IRM was significantly more impermeable than Fermit-N and Cavit G (10). Balto (6) reported that IRM allowed microbial leakage after 10 days, while Cavit and Dyract showed leakage after 2 weeks.

In cases with post preparation, microbial colonization may occur because of material leakage, restoration lost, fracture, or recurrent dental caries. Barrieshi et al. (14) evaluated bacterial leakage of mixed anaerobic microorganisms in obturated canals after post space preparation for 90 days, and found that bacterial penetration occurred after 48 to 84 days. Alves et al. (13) found that endotoxin and mixed bacterial leakage occurred in root canals with post-preparation after 23 and 62 days, respectively. Siqueira et al. (26) reported that root canals filled with calcium hydroxide plus saline solution and calcium hydroxide plus paramonochlorophenol and glycerin showed entire recontamination after 15 and 16 days, respectively. Zucco (30) evaluated the coronal leakage in root canals filled with AH Plus, Sealer 26, and Endofill sealers after post preparation through exposure to artificial saliva contaminated by *S. aureus*. AH Plus sealer showed bacterial leakage after 50 days, while Sealer 26 and Endofill showed leakage after 54 and 56 days, respectively. AH Plus showed greater ability to resist to coronal leakage when compared to the other sealers.

Although the findings of this *in vitro* assay should not be directly extrapolated to the clinics, the present study reinforces some clinical considerations to achieve ideal performance of coronal restorations and endodontic treatment success. The results suggest that, as soon as possible, a definitive coronal restoration should be placed after root canal treatment. However, further research is required to support the clinical application of these findings.

Conclusions

Under the experimental conditions, all tested temporary restorative materials (Coltosol, IRM, Vidrion R) did not prevent microbial microleakage over the 90-day study period. The use of CHP dressing in the prepared post space did not influence sealing ability.

References

1. Hommez GM, Coppens CR, De Moor RJ. Periapical health related to the quality of coronal restorations and root fillings. *Int Endod J* 2002;35:680-89.
2. Kirkevang LL, Orstavik D, Hørsted-Bindslev P, Wenzel A. Periapical status and quality of root fillings and coronal restorations in a Danish population. *Int Endod J* 2000;33:509-15.
3. Ray HA, 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. Saunders WP, Saunders EM. Coronal leakage as a cause of failure in root canal therapy: a review. *Endod Dent Traumatol* 1994; 10:105-8.
5. Tronstad L, Asbjørnsen K, Doving L, Pedersen I, Eriksen HM. Influence of coronal restorations on the periapical health of endodontically treated teeth. *Endod Dent Traumatol* 2000; 16:218-21.
6. Balto H. An assessment of microbial coronal leakage of temporary filling materials in endodontically treated teeth. *J Endod* 2002; 28:762-64.
7. Barkhordar RA, Stark MM, Calif SF. Sealing ability of intermediate restorations and cavity design used in endodontics. *Oral Surg Oral Med Oral Pathol* 1990;69:99-101.
8. Beach CW, Calhoun JC, Bramwell JD, Hutter JW, Miller GA. Clinical evaluation of bacterial leakage of endodontic temporary filling materials. *J Endod* 1996;22:459-62.
9. Deveaux E, Hildelbert P, Neut C, Romond C. Bacterial microleakage of Cavit, IRM, TERM, and Fermit: a 21-day in vitro study. *J Endod* 1999;25:653-59.
10. Jacquot BM, Panighi MM, Steinmetz P, G'Seel C. Evaluation of temporary restoration microleakage by means of electrochemical impedance measurements. *J Endod* 1996;22:586-89.
11. Pai S, Yang S, Sue W, Chueh L, Riviera EM. Microleakage between endodontic temporary restorative materials placed at different times. *J Endod* 1999;25:453-56.
12. Abramovitz I, Tagger M, Tamse A, Metzger Z. The effect of immediate vs. delayed post space preparation on the apical seal of a root canal filling: a study in an increased-sensitivity pressure-driven-system. *J Endod* 2000;26:435-39.
13. Alves J, Walton R, Drake D. Coronal leakage: Endotoxin penetration from mixed bacterial communities through obturated, post-prepared root canals. *J Endod* 1998;24:587-91.
14. Barrieshi KM, Walton RE, Johnson WT, Drake DR. Coronal leakage of mixed anaerobic bacteria after obturation and post space preparation. *Oral Surg Oral Med Oral Pathol* 1997;84: 310-14.
15. Gish SP, Drake DR, Walton RE, Wilcox L. Coronal leakage: bacterial penetration through obturated canals following post preparation. *J Am Dent Assoc* 1994;125:1369-72.
16. Karapanou V, Vera J, Cabrera P, White RR, Goldman M. Effect of immediate and delayed post preparation on apical dye leakage using two different sealers. *J Endod* 1996;22:583-85.
17. Metzger Z, Abramovitz R, Abramovitz I, Tagger M. Correlation between remaining length of root canal fillings after immediate post space preparation and coronal leakage. *J Endod* 2000; 26:724-28.
18. Ravanshad S, Torabinejad M, Iran S, Linda L. Coronal dye penetration of the apical filling materials after post space preparation. *Oral Surg Oral Med Oral Pathol* 1992;74:644-47.
19. Wu MK, Pehlivan Y, Kontakiotis EG, Wesselink PR. Microleakage along apical root fillings and cemented posts. *J Prosthet Dent* 1998;79:264-69.
20. Chailertvanitkul P, Saunders WP, Saunders EM, Mackenzie D. An evaluation of microbial coronal leakage in the restored pulp chamber of root-canal treated multirrooted teeth. *Int Endod J* 1997;30:318-22.
21. Haddix JE, Mattison DG, Shulman AC, Pink EF. Post preparation techniques and their effect on the apical seal. *J Prosthet Dent* 1990;64:515-19.
22. Heling I, Gorfil C, Slutzky H, Kopolovic K, Zalkind M, Slutzky-Goldberg I. Endodontic failure caused by inadequate restorative procedures: review and treatment recommendations. *J Prosthet Dent* 2002;87:674-78.
23. Kersten HW, Moorer WR. Particles and molecules in endodontic leakage. *Int Endod J* 1989;22:118-24.
24. Miletic I, Prpic-Mehicic G, Marsal T, Tambicandrasevic A, Plesko S, Zarlovic Z, Anic I. Bacterial and fungal microleakage of AH 26 and AH plus root canal sealers. *Int Endod J* 2002;35:428-32.
25. Shipper G, Orstavik D, Teixeira FB, Trope M. An evaluation of microbial leakage in roots filled with a thermoplastic synthetic polymer-based root canal filling material (Resilon). *J Endod* 2004; 30:342-47.
26. Siqueira-Jr JF, Lopes HP, Uzeda M. Recontamination of coronally unsealed root canals medicated with camphorated paramonochlorophenol or calcium hydroxide pastes after saliva challenge. *J Endod* 1998;24:11-4.
27. Swanson K, Madison S. An evaluation of microleakage in endodontically treated teeth. Part I. Time periods. *J Endod* 1987; 13:56-9.
28. Torabinejad M, Ung B, Kettering JD. In vitro bacterial penetration of coronally unsealed endodontically treated teeth. *J Endod* 1990;16:566-69.
29. Wu MK, Wesselink PR. Endodontic leakage studies reconsidered. Part I. Methodology, application and relevance. *Int Endod J* 1993;26:37-43.
30. Zucco LR. Avaliação da infiltração coronária em canais obturados e preparados para pinos [dissertation]. Canoas (RS): Universidade Luterana do Brasil; 2001.