International Endodontic Journal

doi:10.1111/j.1365-2591.2011.01852.x

Bacterial leakage in root canals filled with conventional and MTA-based sealers

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Abstract

Oliveira ACM, Tanomaru JMG, Faria-Junior N, Tanomaru-Filho M. Bacterial leakage in root canals filled with conventional and MTA-based sealers. *International Endodontic Journal*, **44**, 370–375, 2011.

Aim To evaluate bacterial leakage after filling root canals with several endodontic sealers, including MTA-based materials.

Methodology One hundred and thirty single-rooted extracted human teeth were randomly divided into experimental groups ($n = 15$) and two control groups ($n = 5$). Six root canal sealers were namely: AH Plus (AHP), Sealer 26 (S26), Epiphany SE (ESE), Sealapex (SEL), Active GP (AGP), Endofill (EDF), and two MTA-based sealers were namely: Endo CPM Sealer (CPM) and MTA-based sealer (MTAS, MTA Sealer). Teeth in the control groups were either filled with no sealer or made completely impermeable. Root canals were prepared and filled with either gutta-percha and one of the sealers or with Resilon and Epiphany SE. Teeth were sterilized by ethylene oxide prior to the bacterial leakage experiments using *Enterococcus faecalis*. Leakage was evaluated every 24 h for 16 weeks. Data were analysed by the Kaplan–Meier, Kruskal–Wallis and Dunn tests at 5% significance.

Results Control groups had either immediate leakage or no leakage. During 120 days, significantly more leaking samples were detected for AGP, CPM and MTAS ($P < 0.05$). The best sealing ability was observed for AH Plus and Sealapex ($P < 0.05$).

Conclusions All sealers evaluated allowed bacterial leakage. The MTA-based sealers had the most leakage.

Keywords: bacterial leakage, endodontic sealer, MTA, root canal filling material.

Received 1 June 2010; accepted 21 December 2010

Introduction

The sealing ability of root filling materials is important for success in root canal treatment. Ideally, the root filling material should seal the root canal system and favour tissue repair (Salz *et al.* 2009). Apical leakage is regarded as a major factor leading to post-treatment disease as is coronal leakage (Adib *et al.* 2004, Siqueira Júnior *et al.* 2005).

Coronal leakage may occur due to voids or loss of restorative material, allowing the root filling material to come into contact with oral fluids (Siqueira Junior *et al.* 1999, Tselnik *et al.* 2004). Among several methods of evaluation of the sealing ability of endodontic materials, bacterial leakage experiments provide biologically and clinically relevant information (Timpawat *et al.* 2001, Shipper & Trope 2004, Pinheiro *et al.* 2009).

AH Plus is an epoxy resin-based endodontic material with good sealing ability (Timpawat *et al.* 2001, Kopper *et al.* 2003, Carvalho-Junior *et al.* 2007, Saleh *et al.* 2008). Sealer 26 is another resin-based material containing calcium hydroxide, and has also demonstrated good sealing ability against bacterial leakage (Siqueira Junior *et al.* 1999).

Sealapex contains calcium oxide and has the ability to induce hard tissue formation at the apex after root canal obturation (Holland & Souza 1985). The barium sulphate in its formulation has been replaced recently with bismuth trioxide, leading to a marked improvement in radiopacity (Tanomaru-Filho *et al.* 2008). Other physical and chemical properties of this sealer are yet to be evaluated.

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Epiphany SE (Self-Etch) is a component in the Epiphany/Resilon system, developed with the goal of promoting better adhesion between the filling materials and the root canal walls. Epiphany contains several methacrylates in its formulation (Ungor *et al.* 2006) and has been recently replaced by Epiphany SE, which does not require a primer.

Due to their potential adhesion to the root canal walls, glass–ionomer-based materials have also been proposed (Weiger *et al.* 1995). The Activ GP system (Brasseler, Savannah, GA, USA) includes glass–ionomer-coated gutta-percha cones and a glass–ionomer-based sealer. In terms of apical leakage, this material behaved similarly to AH Plus after vertical condensation (Monticelli *et al.* 2007).

MTA, a Portland cement-based material (Camilleri 2010), has been generally used in Endodontics for treating root perforations, pulp capping, pulpotomy, and root-end filling. Newer MTA-based cements, such as Endo CPM Sealer, have been developed for use as root canal sealers and have been reported to have good biological properties (Gomes-Filho *et al.* 2009).

An experimental sealer containing Portland cement, radiopacifying agent, additives and appropriate vehicles was developed for use as endodontic filling material. The evaluation of its properties in terms of sealing ability against bacterial leakage in comparison of traditional sealers is important. The null hypothesis was that this formulation of root canal sealer would provide a seal similar to other conventional sealers.

Materials and methods

Human teeth with a single straight root canal were obtained from the Human Teeth Bank at the School of Dentistry of Araraquara, Brazil. The length of the root canal was established using a size 15 K-file up to the apical foramen. Only teeth with apical foramina having diameters smaller or equal to a size 15 K-file were selected. The root canals were instrumented up to 1 mm short of the foramen with a size 40 K-file, and then stepped-back to a size 60 K-file under irrigation with 1% sodium hypochlorite. The canals were then irrigated with 17% EDTA agitated for 3 min, followed by irrigation with saline. The canals were dried with size 40 paper points.

The root canal sealers used, as well as their formulations and manufacturers, are listed in Table 1. All materials were manipulated according to the manufacturer's instructions and inserted into the canals with a size 40 K-file up to the working length. After that, a size 40 gutta-percha cone (Tanari Ind. Ltda. Manacapuru, AM, Brazil) or Resilon cone (Pentron Clinical Technologies, LLC., Wallingford, CT, USA) coated with cement was inserted and accessory gutta-percha cones (Tanari Ind. Ltda. Manacapuru, AM, Brazil) were placed with the aid of finger spreaders (Dentsply Maillefer, Ballaigues, Switzerland) under active lateral condensation.

Epiphany SE sealer was used with Resilon cones, and Activ GP was used with glass–ionomer-coated gutta-percha cones. In the Epiphany SE/Resilon group, after sealing and removing the excess cement, the materials were light-cured for 40 s.

The five specimens in the positive control group were filled with the core material without the use of sealer. The root canals of the specimens in the negative control group were filled with gutta-percha or Resilon and sealer. All teeth were radiographed until satisfactory filling of the root canal was confirmed. Then, the specimens were placed in a moist environment at 37 °C for 7 days for the materials to set.

After that period, the external surfaces of all specimens were made impermeable with two layers of epoxy adhesive (Araldite de presa rápida, Ciba-Geigy AS, Taboão da Serra, SP, Brazil), except for the area 1 mm around the apical foramen. Specimens were then mounted to an apparatus with polypropylene microtubes (Eppendorf®). The interface between the roots and the microtubes was sealed with adhesive and each assembly was sterilized by ethylene oxide gas (ACECIL, Central de Esterilização Com. Ind. Ltda., Campinas, SP, Brazil) at 56 °C.

After that, each assembly was positioned so that the apical portions of the roots were immersed in BHI (Brain Heart Infusion, Difco Laboratories-Becton Dickinson and Company, Franklin Lakes, NJ, USA). The interface of the microtubes with the glass tubes containing BHI was sealed with adhesive. Each assembly was labelled and kept in an oven at 37 °C for 4 days in order to confirm the sterility of the assembly.

For the coronal leakage assays, a standard strain of *E. faecalis* (ATCC 29212) was used. Previously to testing, the *E. faecalis* counts in the BHI were determined by decimal dilutions. Aliquot portions were plated on the surface of trypticase soy agar (Difco Laboratories, Becton Dickinson and Company, Franklin Lakes, NJ, USA) and incubated at 37 °C for 24 h. After the incubation period, the number of colony forming units (CFU mL^{-1}) was determined.

For assessment of bacterial leakage, 500 μL aliquots of standard *E. faecalis* were transferred to the upper portion of the Eppendorf® microtubes contacting the

Table 1 Constituents and manufacturers of the root canals sealers

Cement	Composition	Manufacturer
AH Plus (AHP)	Paste A: epoxy resin; calcium tungstate; zirconium oxide; aerosil and iron oxide Pasta B: aminoadamantane; N,N′-dibenzyl-5-oxa-nonandiamine-1,9; TCD-diamine; calcium tungstate; zirconium oxide; aerosil, and silicone oil	Dentsply/De Trey, Konstanz, Germany
Sealer 26 (S26)	Powder: bismuth trioxide; calcium hydroxide; hexamethylenetetramine; titanium dioxide; bisphenol epoxy resin	Dentsply, Petrópolis, RJ, Brazil
Epiphany SE (ESE)	UDMA, PEGDMA, EBPADMA, BISGMA resins and methacrylates; silane-treated barium borosilicate glass; barium sulfate; silica; calcium hydroxide; bismuth oxychloride; peroxides; photoinitiator; stabilizers and pigments	Pentron Clinical Technologies, LLC., Wallingford, CT, USA
Sealapex (SEL)	Base: calcium oxide, zinc oxide, sulfonamides, and silica Catalyst: bismuth trioxide, polymethyl methacrylate, methyl salicylate, titanium dioxide, silica, pigments, isobutyl salicylate	Kerr Corp., CA, USA
Activ GP (AGP)	Powder: calcium silicate (silica; cryolite; aluminum fluoride; aluminum phosphate) Liquid: polyacrylic acid	Brasseler USA, Dental Instrumentation, USA
Endofill (EDF)	Powder: zinc oxide; hydrogenated resin; bismuth subcarbonate; barium sulfate, and sodium borate. Liquid: eugenol and sweet almond oil	Dentsply Indústria e Comércio Ltda, Petrópolis, RJ, Brazil
Endo CPM Sealer (CPM)	Mineral trioxide aggregate: silicon dioxide; calcium carbonate; bismuth trioxide; barium sulfate; propylene glycol alginate; propylene glycol; sodium citrate; $CaCl_2$; active ingredients	EGEO S.R.L. under license of MTM Argentina S.A., Buenos Aires, Argentina
MTA Sealer (MTAS)	Portland cement, radiopacifying agent, additives, vehicle	Araraquara Dental School, UNESP, Brazil

coronal portion of the filling materials. After every 7 days during the experimental period, the BHI inoculated with *E. faecalis* was replaced with a new 500 μL aliquot of sterile BHI. The aliquot removed was tested to confirm bacterial viability.

Samples were observed daily for 120 days, and leakage was detected by turbidity of the BHI medium in contact with the apex. When turbidity of the medium was observed, confirmation of cell morphology was carried out by Gram stain. Another portion of turbid BHI was plated on TSA medium to detect growth of *E. faecalis* and observe colony morphology. The number of leaking samples for each group at different time intervals was observed. Data were subjected to the Kruskal–Wallis, Kaplan–Meier tests, and to Dunn paired comparisons at 5% significance.

Results

From the initial 130 roots, three specimens were discarded during the apparatus sterility observation period. All specimens in the positive control group had turbidity of the medium within 24 h, while none in the negative control group became turbid. During the experimental period, 93 specimens had bacterial leakage (Table 2). In all cases, the inoculum was confirmed to contain *E. faecalis*.

AH Plus and Sealapex had, respectively, 60% and 73.3% cases of bacterial leakage during the experimental period, with statistically significant difference ($P < 0.05$) compared with Activ GP, Endo CPM Sealer, and MTAS. Sealer 26, Epiphany SE, and Endofill had intermediate and similar results. Activ GP, Endo CPM Sealer, and MTAS allowed, respectively, 100%, 86.6%, and 92.3% of bacterial leakage.

Discussion

The culture medium of specimens filled without the use of sealer became turbid within the first 24 h, confirming the importance of endodontic sealers when filling root canals (Saunders *et al.* 2004, Shipper & Trope

Table 2 Number and percentage of specimens with bacterial leakage during the experimental period

Material	Period (days) 1–30 *n*	%	31–60 *n*	%	61–90 *n*	%	91–120 *n*	%	1–120 *n*	%	Total *n*
AHP[(a,b)]	5	33.33	2	13.33	1	6.66	1	6.66	9	60.00	15
S26[(b)]	8	53.33	1	6.66	0	0	1	6.66	10	66.66	15
ESE[(b)]	4	26.66	5	33.33	2	13.33	1	6.66	12	80.00	15
SEL[(a,b)]	7	46.66	0	0	1	6.66	3	20.00	11	73.33	15
AGP[(b,c)]	6	42.85	5	35.71	3	21.42	0	0	14	100	14
EDF[(b)]	8	53.33	1	6.66	2	13.33	1	6.66	12	80.00	15
CPM[(b,c)]	9	60.00	1	6.66	2	13.33	1	6.66	13	86.66	15
MTAS[(b,c)]	8	61.53	2	15.38	1	7.69	1	7.69	12	92.30	13

Materials with the same letter (a, b) were not significantly different ($P > 0.05$).

2004). In the present study, evaluation was carried out at 120 days, the majority of the specimens showed bacterial leakage. Coronal leakage was detected in 20% of the specimens within 3 days. Barthel *et al.* (1999) observed bacterial leakage in 57% of the samples after 3 days. This divergence in bacterial leakage percentages may be due to the anatomical complexity of the root canal systems.

AH Plus and Sealapex were the most resistant against bacterial leakage. The favourable results for AH Plus may be related to its flowability and dimensional stability, which led to a reduction in marginal leakage (Timpawat *et al.* 2001, Kopper *et al.* 2003, Carvalho-Junior *et al.* 2007). Timpawat *et al.* (2001) also verified that AH Plus had good resistance to leakage, which suggests better adaptation of this material to dentine walls. Saleh *et al.* (2008) reported that AH Plus had less leakage when the smear layer was removed, highlighting the importance of using EDTA. De-Deus *et al.* (2006) analysed bacterial leakage in canals filled with four different endodontic cements and reported that AH Plus had the best performance.

Sealapex leaked less than Activ GP, Endo CPM Sealer, and MTAS. The newer formulation of this material was used, in which barium sulphate was replaced with bismuth trioxide. Yucel *et al.* (2006) did not observe any differences between AH Plus, AH 26, Sealapex, and Ketac-Endo in terms of bacterial leakage.

Sealer 26 is an epoxy resin-based cement composed of bismuth oxide, calcium hydroxide, and epoxy resin. In this study, this material had intermediate resistance to bacterial leakage. Siqueira *et al.* (2001b) reported satisfactory sealing ability when Sealer 26 was used in root canal fillings. Moreover, it has been demonstrated that Sealer 26 is able to prevent bacterial leakage when used both as a root canal sealer (Hollanda *et al.* 2009) and in root-end filling (Siqueira *et al.* 2001a).

Epiphany SE had intermediate results for resistance against bacterial leakage. Lyons *et al.* (2009) evaluated the sealing ability against microleakage using *Streptococcus mutans*, and reported inadequate results for the Resilon system in terms of apical seal. Few studies focusing on the properties of Epiphany SE have been carried out. De-Deus *et al.* (2009) observed better adhesion of AH Plus® to gutta-percha than the Resilon/Epiphany® and Resilon/Epiphany SE® associations, reporting bacterial leakage results that agree with the present study.

Endofill also showed intermediate resistance to bacterial leakage in the present experiment. Regarding adhesion properties, Lee *et al.* (2002) observed that AH 26, an epoxy resin-based cement, has better adhesion to gutta-percha than zinc oxide and eugenol-based materials. AH Plus and Sealer 26, materials evaluated in this study, are also epoxy resin-based.

Similarly to the Resilon/Epiphany system, adhesion of Active GP to the root canal walls is described as forming a monoblock (Fransen *et al.* 2008). However, Activ GP had less resistance to bacterial infiltration when compared with AH Plus and Sealapex. Friedman *et al.* (1995), after a clinical study, supported the use of glass–ionomer cement (Ketac-Endo) as an acceptable endodontic sealer. Monticelli *et al.* (2007) employed a bacterial leakage model using *S. mutans*, and observed that root canals filled with Activ GP and a single cone suffered more leakage than AH Plus.

Currently, MTA and Portland cement-based materials have been proposed for use in root canal fillings (Jacobovitz *et al.* 2009, Bernardes *et al.* 2010). Endo CPM Sealer, one commercially available material is able to release calcium and hydroxyl ions (Tanomaru-Filho *et al.* 2009), and is biocompatible (Gomes-Filho *et al.* 2009). The antimicrobial characteristics of the sealers used in bacterial leakage studies may influence the results. MTA-based materials have some antibacterial and antifungal properties (Parirokh & Torabinejad 2010). However, Endo CPM Sealer resulted in a higher ratio of bacterial leakage compared with AH Plus and Sealapex.

Conclusions

AH Plus and Sealapex presented less coronal leakage ($P < 0.05$), while Active GP and the MTA-based materials (Endo CPM Sealer and MTAS) were less resistant to coronal leakage.

Acknowledgement

We thank Ms. Hiroko Yamamoto for expert secretarial assistance. Financial support: FAPESP 07/08563-3.

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