doi:10.1111/j.1365-2591.2007.01253.x

Ex vivo microscopic assessment of factors affecting the quality of apical seal created by root-end fillings

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Abstract

Tobón-Arroyave SI, Restrepo-Pérez MM, Arismendi-Echavarría JA, Velásquez-Restrepo Z, Marín-Botero ML, García-Dorado EC. *Ex vivo* microscopic assessment of factors affecting the quality of apical seal created by root-end fillings. *International Endodontic Journal*, **40**, 590–602, 2007.

Aim (i) To evaluate the incidence of microcracks around root-end preparations completed with ultrasonic tips and their relationship with the root filling technique and thickness of surrounding dentine. (ii) To investigate the effect of rapid exposure to a water-soluble dye of Intermediate Restorative Material (IRM), Super Ethoxy-benzoic Acid (sEBA) and Mineral Trioxide Aggregate (MTA), on the marginal adaptation and microleakage of root-end fillings. (iii) To describe the microstructure of the surface of root-end filling materials.

Methodology Ninety-two single-rooted teeth were divided into two groups ($n = 46$) according to the root canal instrumentation/filling techniques. Group 1 consisted of specimens in which canal preparation was completed using a crown-down technique and then filled with the Thermafil system (TF group); Group 2 consisted of specimens in which canal preparation was completed using a step-back technique and lateral condensation (LC group). Following root-end resection and ultrasonic cavity preparation, the samples were further divided into three subgroups ($n = 24$) for root-end filling with IRM, sEBA or MTA. The ultrasonic preparation time was recorded. Eight teeth were kept as positive and 12 as negative controls. Following immersion in Indian ink for 7 days, all resected root surfaces were evaluated for the presence of microcracks and the cross-sectional area of root-end surface and root-end filling were measured to evaluate the thickness of the dentinal walls. Thereafter, the samples were sectioned longitudinally so as to assess the depth of dye penetration and marginal adaptation of root-end fillings. Negative controls longitudinally sectioned were used to describe microstructural characteristics of the root-end filling materials using scanning electron microscopic (SEM) techniques.

Results Although the thickness of dentinal walls between groups 1 and 2 was similar, the ultrasonic preparation time and number of microcracks were significantly higher ($P < 0.001$) in the TF group. Both groups had a significant correlation between microcracks and ultrasonic preparation time ($P < 0.001$). sEBA and IRM had better adaptation and less leakage compared with MTA. A SEM analysis displayed microstructural differences between the root-end filling materials.

Conclusion Microcracks can occur independently of the thickness of dentinal walls and may be associated with the prolonged ultrasonic preparation time required for the removal of the root filling during root-end cavity preparation. Although sEBA and IRM had better behaviour than MTA regarding microleakage and marginal adaptation, it is possible that exposure of MTA to a water-soluble dye before achieving full set and its porous microstructure contributed to the results.

Keywords: marginal adaptation, microcracks, microleakage, microstructure, root-end filling materials.

Received 18 April 2006; accepted 8 January 2007

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Introduction

One of the functions of a root-end filling material during periradicular surgery is to provide an apical seal at the root apex, thereby sealing the periradicular tissues from egress of bacteria and bacterial by-products from infected root canals. This goal is usually achieved by preparing a root-end cavity and then filling it with a biocompatible material (Abedi *et al.* 1995). Nevertheless, there are various factors that influence the sealing ability of the materials, which include microcrack formation (De Bruyne & De Moor 2005), microleakage (Yaccino *et al.* 1999), marginal adaptation (Peters & Peters 2002, Gondim *et al.* 2003) and the microstructure of filling materials (Yoshikawa *et al.* 1997).

The development of ultrasonic devices has allowed improvement in the overall quality of the root-end cavity (Kim 1997). However, the formation of microcracks during and/or following ultrasonic root-end preparation is a concern (Saunders *et al.* 1994, Layton *et al.* 1996, Von Arx & Walker 2000, Navarre & Steiman 2002, De Bruyne & De Moor 2005). It has been stated that microcrack formation is a consequence of the power of the unit, the time of application, the presence of initial microcracks and the thickness of surrounding dentine (Abedi *et al.* 1995). Furthermore, the management of the resected root-end may be influenced by the nature of the root filling (Taschieri *et al.* 2004) that itself could be associated with microcrack formation. If the canal is filled with gutta-percha, root-end cavity preparation is relatively straightforward, but when a plastic carrier is present considerable effort could be required to remove it during root-end cavity preparation (Baker & Oguntebi 1990, Taschieri *et al.* 2004). However, the relationship between plastic core removal and microcrack formation during periradicular surgery is unknown.

Another approach to assess the sealing ability of a root-end filling material is to determine the quality of marginal adaptation. Although there is increasing evidence suggesting that marginal adaptation is of great importance in the success of periradicular surgery (Stabholz *et al.* 1985, Peters & Peters 2002, Gondim *et al.* 2003), some authors have expressed doubt about the potential relation between them (Abdal & Retief 1982).

Many of the studies regarding sealing ability of root-end filling materials have focused on the assessment of microleakage. Microleakage, defined as the passage of bacteria, fluids and chemical substances between the root structure and filling material, has been evaluated *ex vivo* by numerous techniques (Yaccino *et al.* 1999), which include passive penetration of dyes, such as Indian ink (Andelin *et al.* 2002, Davis *et al.* 2003), fuchsin (Gagliani *et al.* 1998), rhodamine (Kubo *et al.* 2005) and methylene blue (Aqrabawi 2000, Reeh & Combe 2003), fluid perfusion tests (Taschieri *et al.* 2004, De Bruyne *et al.* 2005, Gondim *et al.* 2005), bacterial penetration models (Adamo *et al.* 1999, Scheerer *et al.* 2001) and capillary flow porometry (De Bruyne *et al.* 2005, 2006). However, the results of the many studies reported are often conflicting. It must be considered that the results of laboratory studies are dependent on the type of experimental model used and the difficulty in the extrapolating the dynamic nature of the leakage pattern between the tooth and the periradicular tissues (Scheerer *et al.* 2001).

Scanning electron microscopic (SEM) analysis appears to be an efficient and acceptable method of examining features, such as surface topography, porosity and particle size of dental materials. Several publications have discussed the marginal adaptation of root-end filling materials (Fitzpatrick & Steiman 1997, Peters & Peters 2002, Gondim *et al.* 2003, Shipper *et al.* 2004) using SEM analysis, but only a few have specifically assessed the porosity of MTA (Fridland & Rosado 2003) and microstructure of zinc oxide–eugenol (ZOE) cements (Yoshikawa *et al.* 1997). However, none have comprehensively discussed the sealing ability of these materials, based upon a SEM analysis of their microstructures.

Since its earliest report in the literature, MTA has shown superior sealing properties to the other root-end filling materials, including IRM and sEBA, with respect to marginal adaptation and microleakage (Shipper *et al.* 2004). However, MTA has also been criticized in relation to the requirements of an ideal material in two regards: difficulty to use and very slow setting time (Lee 2000), which may contribute to a greater degree of leakage (Reeh & Combe 2003), surface disintegration (Yatsushiro *et al.* 1998, Davis *et al.* 2003) and loss of marginal adaptation and continuity (Peters & Peters 2002) of the material.

The aims of this study were: (i) to evaluate the incidence of microcracks around root-end preparations completed with ultrasonic tips and their relationship with the root filling technique and thickness of surrounding dentine; (ii) to investigate the effect of rapid exposure to a water-soluble dye of IRM, Super EBA and MTA, on the marginal adaptation and microleakage of root-end fillings; and (iii) to describe the microstructure of the surface of root-end filling materials.

Materials and methods

Tooth selection, storage and initial preparation

A total of 92 single-rooted maxillary and mandibular human teeth freshly extracted for reasons that were not related to this study were selected. The study conformed to the ethical guidelines of the Helsinki declaration and was approved by the Institutional Research Ethics Board (CIFO). Samples with immature apices, root fractures, resorption, root caries, deep-root concavities and root canal treatment were excluded. The teeth were radiographed to determine the existence of a single relatively straight canal and they were excluded if radiographs demonstrated multiple canals or pulpal obliteration. After extraction, the teeth were fixed in a Karnowsky solution (2.5% glutaraldehyde + 2.0% paraformaldehyde) diluted in a Sörensen phosphate buffer (pH 7.4; 0.1 mol L^{-1}) and kept at 4 °C in a refrigerator for <4 weeks before use. Then, to remove periodontal remnants, the teeth were placed into 5.25% NaOCl for 10 min and cleaned using ultrasonic and hand scaling. The crowns were sectioned at the cemento-enamel junction using a low-speed diamond saw (Isomet®; Buehler Ltd, Lake Bluff, NY, USA) with constant water irrigation. All specimens were screened under ×50 magnification using a Stemi 2000® stereomicroscope (Carl Zeiss, Oberkochen, Germany) and those teeth exhibiting cracks or fractured apices were discarded. Thereafter, the roots were immersed in 0.9% NaCl solution containing 0.02% thymol (Merck, Darmstadt, Germany) to inhibit microbial growth in individual numbered containers at 4 °C. Figure 1 shows the flow diagram of sample progress through the phases of the experimental study.

Root canal preparation

Root canal treatment was performed by a single operator (ZV) while holding the specimens in moist gauze. The canals were instrumented to within 1 mm of the root apex with 2.5% NaOCl irrigation between each file. The samples were divided randomly using a computer-generated randomization code into two groups ($n = 46$) according to the root canal instrumentation and filling techniques as follows.

TF group

Canal preparation was completed using a crown-down technique, and Ni–Ti ProFile® 0.06 Taper Series 29 rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland). The instruments were mounted in a reduction handpiece with a 16 : 1 ratio powered by an electric motor ATR-Tecnika® (Dentsply Maillefer, Tulsa Dental, Tulsa, OK, USA) set at 300 rpm. All root canals were dried with paper points and then filled with a carrier obturator (Thermafil® Dentsply Maillefer) and root canal sealer (Top Seal® Dentsply Maillefer). Verifiers (Dentsply Maillefer) were used to confirm the appropriate size for filling the canal.

LC group

Root canals were instrumented using a passive step-back technique, with a minimum master file size of 30 (Flexofile®; Dentsply Maillefer). After instrumentation, the canals were dried with sterile paper points, and filled with gutta-percha and root canal sealer (Top Seal® Dentsply Maillefer) using a cold lateral compaction technique.

The coronal opening for each canal was sealed with a resin modified glass–ionomer cement (Vitremer®; 3M Health Care, St Paul, MN, USA) and subsequently, all teeth were stored at 37 °C and 100% humidity to allow complete setting of the sealer.

Root-end preparation

All root-end procedures were performed by a single investigator (ST) using high-resolution surgical binocular loupes (×6.0 magnification) fitted to a fibre optic headlight (Heine Optotechnik®, Herrsching, Germany). Approximately 3 mm of the root apex was resected (0° bevel) using sterile water-cooled diamond finishing burs (Perioset® No. 575; Intensiv, Viganello, Switzerland) at high speed. Thereafter, each root-end surface was inspected by a trained research associate (MR) at ×40 magnification using a stereomicroscope to ensure that no cracks were present after the root-end resection. Preparation of the root-end cavities was performed using diamond ultrasonic retrotips (DFy-908 double-angled files with 0.8-mm shanks, ENAC-OE505S®; Osada Electric Co. Ltd, Los Angeles, CA, USA) at the intensity prescribed by the manufacturer (power 8 at power mode VIB). A feather-like back-and-forth motion was applied with slight coronal pressure and tap water cooling. The length of the retrotip (3 mm) determined the depth of the preparation and the final diameter was determined by the circumferential movement carried out to complete the entire preparation. The time required to prepare the root-end cavities was recorded. The preparations were sprayed with a water stream for 5 s and then dried with sterile paper points. The teeth

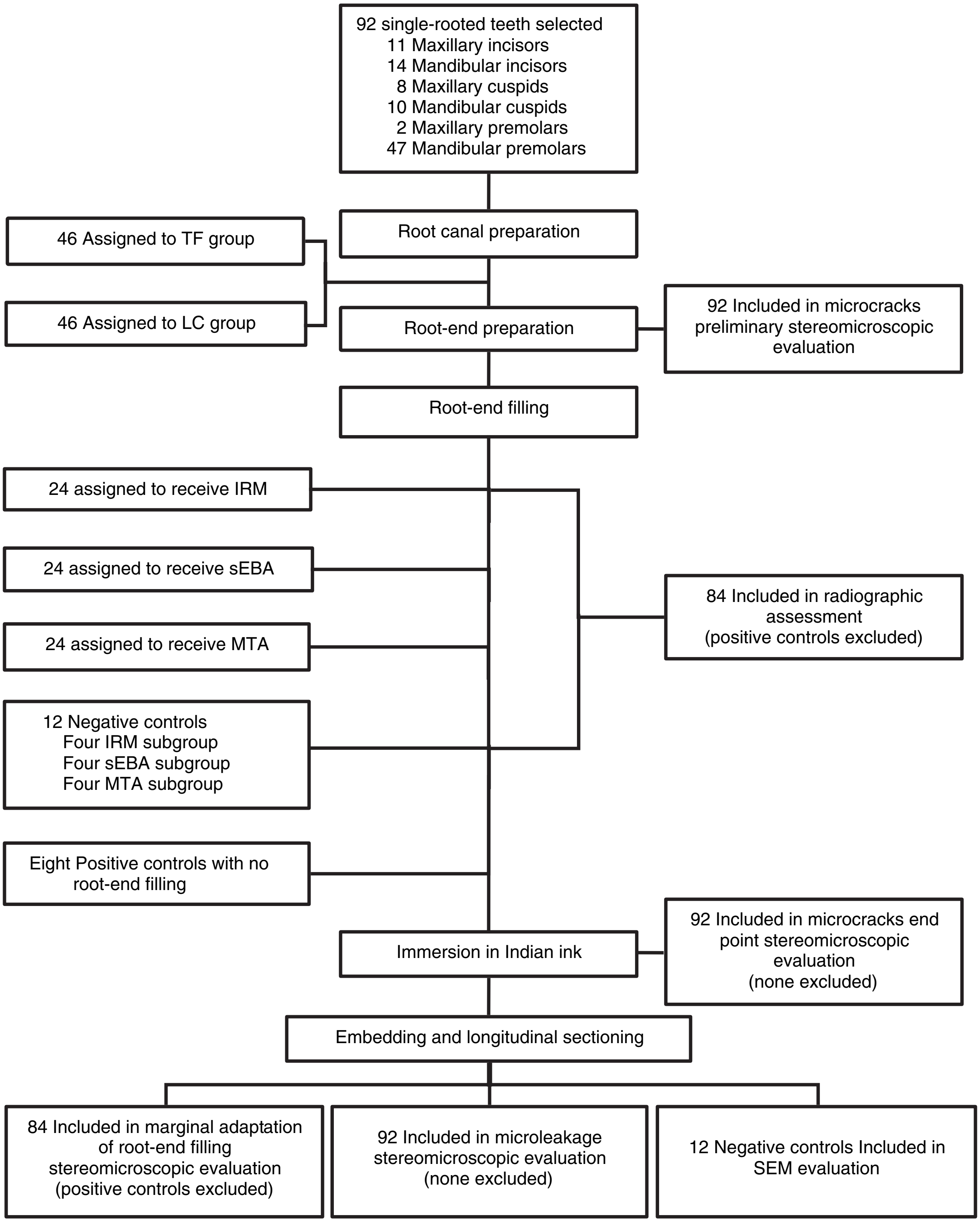

Figure 1 Flow of samples through the trial.

were kept wrapped in moistened gauze during the entire root-end preparation procedure, exposing only the surface of the resected root-end.

After root-end cavity preparation, the resected surfaces were again examined for microcrack formation by the same examiner (MR) under a stereomicroscope at

×40 magnification. The observations were repeated with at least a 1-week interval with a sensitivity (proportion of the roots containing cracks that were correctly diagnosed as having cracks) of 91%, specificity (proportion of the roots without cracks that were correctly diagnosed as not having cracks) of 95% and accuracy (proportion of the diagnoses that agreed with the known root condition) of 93% (Slaton *et al.* 2003).

Root-end filling

The prepared teeth in each main group were again randomly divided into three subgroups ($n = 24$) with respect to the root-end filling material to be used and mixed according to manufacture's recommendations: (i) IRM Caps (Caulk Dentsply; Mildford, DE, USA) placed into the preparation using a Messing® syringe (Union Broach; Moyco Ind., Emigsville, PA, USA), condensed with Buchanan pluggers PLGRF1® (Hu-Friedy, Chicago, IL, USA) and finished with diamond finishing bur (Perioset® No. 575; Intensiv) at high speed after setting. (ii) sEBA fast set (Bosworth; Skokie, IL, USA) placed with a plastic instrument, tamped down with Buchanan pluggers and finished in the same manner as IRM samples. (iii) MTA Tooth Coloured (Pro-Root®; Caulk Dentsply) placed with a plastic instrument, tamped down with Buchanan pluggers and finished with a moistened gauze. After finishing of each root-end filling, the material was allowed to set for 30 min and a new radiograph was taken to verify its uniformity and density. Three layers of nail polish were applied to the external surface of all roots except at the resected root surface.

The negative controls for each subgroup consisted of four teeth prepared and filled with different techniques as described ($n = 12$), but their entire root surfaces were covered with three coats of nail polish. Furthermore, eight instrumented roots were used as positive controls. These samples were prepared as in the experimental groups, except no root-end fillings were placed.

Microcracks, microleakage and marginal adaptation assessment

The samples were submerged in Indian ink (Higgins No. 4415®; Sandford, Bellwood, IL, USA) for 7 days at 37 °C and 100% humidity. Thereafter, the roots were removed, rinsed with distilled water and air dried. The nail polish was removed using 2% acetone. A single calibrated investigator (MR) performed blind assessment of the root-end surface by using an image analyser system (AxioVision 3.1®; Carl Zeiss) fitted to a stereomicroscope. Images were calibrated using a microscope ocular micrometer (Carl Zeiss) as reference, which was put into the computer programme.

To detect any microcracks occurring before and after root-end filling, stained root-end surfaces were evaluated at ×40 magnification. Microcracks were thus categorized based on previously defined criteria (Rainwater *et al.* 2000), as: *intracanal cracks* (those that originate within the canal and extend into the dentine), *extracanal cracks* (those that originate on the root surface and extend into the dentine) and *communicating cracks* (those that extend from the root surface to the canal).

Furthermore, the cross-sectional areas of the root-end surface (R_{es}) and root-end filling (R_{ef}) were measured in mm^2 to obtain the $R_{ef} : R_{es}$ ratio so as to evaluate the thickness of the dentinal walls using the following equation:

$$R_{ef} : R_{es} \text{ ratio} = \frac{R_{ef} \text{ area}}{R_{es} \text{ area} - R_{ef} \text{ area}}$$

After a digital record was taken, the samples were embedded in acrylic resin (Veracril®, New Stetic, Medellín, Colombia) and sectioned longitudinally into two halves using a low-speed diamond saw (Isomet®) with constant water irrigation. Each surface was gently polished for 1 min through 3, 1, and 0.5-μm fine abrasive papers (Diamond Lapping film/PSA®l EMS, Fort Washington, PA, USA) with light finger pressure. The polished surfaces were washed with distilled water, air dried and examined at ×50 magnification to assess the depth of dye penetration and the marginal adaptation of root-end filling materials.

To assess microleakage, the total length of the root-end filling and the depth of dye penetration along the interface of the root-end filling and root canal wall for both halves of each root were recorded in millimetres. The largest measurement for each root was used as the leakage value. As not all of the root-end cavities were exactly 3 mm deep, the dye leakage data were converted to percentages by dividing the length of the leakage by the length of the root-end filling, to nullify the effects of variable lengths.

Marginal adaptation was categorized following a previous description (Peters & Peters 2002), as *continuous margin* (no gap visible between root-end cavity wall and filling material), *overfilled margin* (filling material covers interface between filling and dentine), *underfilled margin* (surface of filling material below level of surface of resected root-end) and *noncontinuous margin* (gap apparent between root-end cavity wall and filling material).

SEM evaluation

All negative controls ($n = 12$) sectioned longitudinally were used to describe microstructural characteristics of the root-end filling materials. The samples were rinsed with distilled water, air dried, mounted on stubs and gold-coated (30 nm thick) with a Desk II® (Denton Vacuum, Moorestown, NJ, USA) ion-sputter coater. Samples were then stored in a desiccating cabinet until evaluated using a JSM-5910 LV® (JEOL Ltd, Tokyo, Japan) electron microscope. The specimens were introduced in the SEM column under 13–20 kV power, maintained at high vacuum conditions (10^{-6} Torr) and observed at ×9500 magnification. A descriptive analysis of the micrographs was performed.

Statistical analysis

Data collected were analysed using SPSS 13.0® (SPSS Inc., Chicago, IL, USA) statistical package. All parameters were tested for normal distribution using the Shapiro–Wilk test. Because the results for each group and subgroup did not follow a normal distribution (except for length of the root-end fillings that showed a pattern conforming to a normal distribution), the variables (excluding the latter) were analysed using nonparametric methods. In the bivariate analysis, chi-squared tests were used to assess the statistical significance of the effects of root canal preparation (LC or TF) and root-end filling on the microcracks and marginal adaptation respectively. To compare the $R_{ef} : R_{es}$ ratio, microcracks per root-end surface, and ultrasonic preparation time with respect to the root canal preparation groups (LC or TF) the Mann–Whitney *U*-test was used. Correlations amongst $R_{ef} : R_{es}$ ratio and ultrasonic preparation time with regard to microcracks were assessed using Spearman's rank correlation coefficient. A parametric one-way analysis of variance (ANOVA) was applied to determine differences amongst continuous data of length of the root-end fillings. The percentages of microleakage related to the root-end filling materials were compared using the Kruskal–Wallis and Mann–Whitney *U*-tests. Statistical significance was established at a value of $P < 0.05$.

Results

Radiographs revealed that all roots were prepared and filled to the appropriate depth. No samples were replaced or excluded from the study because of an improper root-end filling technique. The comparison between the $R_{ef} : R_{es}$ ratio data obtained from the root samples showed values ranging from 0.053 to 0.204 with a median at 0.094 for TF group, similar to the LC group ($P = 0.773$, Mann–Whitney *U*-test), where the $R_{ef} : R_{es}$ ratio was distributed from 0.029 to 0.304 with a median at 0.090. That is, each group comprised roots with similar thickness of the dentinal walls which varied from relatively low $R_{ef} : R_{es}$ ratios (suggestive of solid dentinal walls) to high $R_{ef} : R_{es}$ ratios (suggesting thin dentinal walls).

Microcracks

None of the roots had visible cracks after root-end resection. The time required to prepare the root-end cavities and distribution of microcracks by experimental group are listed in Table 1. The median value of the time required to prepare all cavities in the LC group was significantly less ($P < 0.001$, Mann–Whitney *U*-test) than that required for the TF group. After root-end preparation a total of 34 roots exhibited cracks with varied configurations (Fig. 2a–c). All cracks were confined to the root-end surface with minimal cervical propagation. None of the roots exhibited new cracks after root-end filling. A significantly greater number of roots with cracks ($P = 0.005$, chi-squared test) were observed in TF group.

On the other hand the number of microcracks per root-end surface were distributed from 0 to 6 with a median at 0.5 and a mode of 1 for the TF group, significantly greater ($P < 0.001$, Mann–Whitney *U*-test)

Table 1 Comparison of ultrasonic preparation time and distribution of microcracks by experimental group

Experimental group	Preparation time (min), median (range)	Type of microcracks			Root-ends with cracks *n* (%)
		Intracanal	Extracanal	Communicating	
TF group, $n = 46$	1.3 (0.6–2.9)	24	2	3	24 (52)
LC group, $n = 46$	0.8 (0.3–2.0)	10	1	1	10 (21)

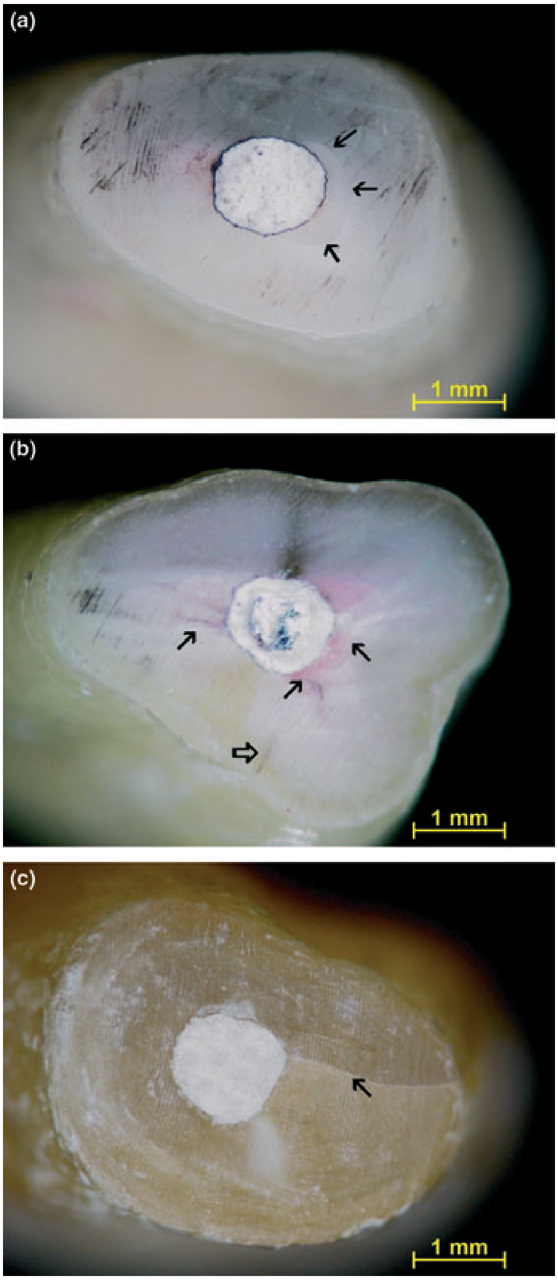

Figure 2 Photomicrographs of resected root surfaces showing microcracks observed after ultrasonic cavity preparation/root-end filling and immersion in Indian ink. (a) Intracanal crack with a semilunar pattern (thin arrows) originated within the canal and extends into dentine (LC group/IRM). (b) Extracanal crack (thick arrow) developed on the root surface and extends into the dentine. Synchronically three intracanal cracks (thin arrows) are also seen in a branching pattern (TF group/sEBA). (c) Communicating crack (thin arrow) extends from the root surface to the canal (TF group/MTA). Original magnification ×40.

than the LC group where the microcracks were distributed from 0 to 3 with a median at 0 and a mode of 0. In addition, although there were significant positive correlations ($P < 0.001$) between the number of microcracks and the ultrasonic preparation time in both TF ($r = 0.915$) and LC ($r = 0.719$) groups, no correlation between the number of microcracks and the $R_{ef} : R_{es}$ ratio ($r = -0.18$, $P = 0.297$) was evident.

Microleakage

No test material was able to prevent microleakage, as dye penetration was found in all 24 MTA samples (100%), 22 IRM specimens (91.6%) and 12 sEBA root-end fillings (50%). The pattern of leakage between MTA and the other two materials was different. The leakage for both IRM and sEBA was confined to a circumferential pattern, whilst MTA showed absorption of dye throughout the root-end filling (Fig. 3a–c). In most samples, the observed leakage extended in the apical to coronal direction, although in some samples minimal dye penetration into the dentinal tubules was also noted. All positive controls showed leakage throughout, whereas the negative controls showed no dye penetration.

Results of the leakage test are summarized in Table 2. Although no significant differences were observed between the length of the root-end fillings ($P = 0.754$, one-way ANOVA), the percentages of leakage were significantly different amongst the three subgroups ($P < 0.001$, Kruskal–Wallis test). A *post hoc* Mann–Whitney *U*-test demonstrated significantly greater values in the MTA samples compared with the other two materials ($P < 0.001$). Conversely, no significant difference ($P = 0.65$) was observed between the IRM and sEBA samples.

Marginal adaptation

Low magnification observation (×50) of the longitudinally sectioned apical segments revealed variation amongst root-end filling materials regarding marginal adaptation (Fig. 3a–c). The three materials exhibited some marginal irregularity. IRM showed the best uniformity of marginal adaptation and the greater number of specimens with a continuous margin (71.4%) whilst sEBA had a greater proportion of overfilled margins (46.4%). Conversely, MTA root-end fillings had an initial 'washing out' of the material before the final set, and exhibited the greatest number of underfilled samples (53.6%) and noncontinuous

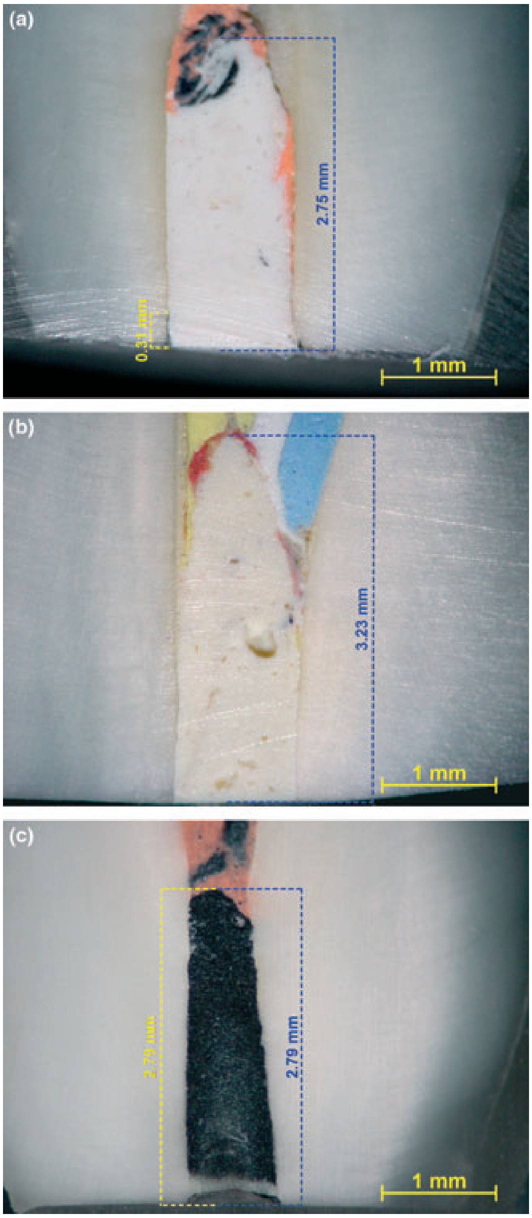

Figure 3 Longitudinal sections of the samples showing representative features of the leakage pattern and marginal adaptation observed for the three root-end filling materials. Dotted lines represent the length of the root-end filling (blue) and the depth of dye penetration (yellow) respectively. (a) Example of an IRM root-end filling (TF group) showing a continuous margin and minimal dye penetration in the apical to coronal direction. (b) Example of a sEBA root-end filling (LC group) with a continuous margin and a complete absence of staining. (c) Example of a MTA root-end filling (TF group) showing an underfilled margin and dye penetration throughout the material until the depth of the cavity preparation. Original magnification ×50.

Table 2 Results of dye leakage evaluation for the root-end filling materials

	Parameters	
Root-end filling	Length of the root-end filling (mm), mean (SD)	Percentage of leakage, median (range)
IRM ($n = 24$)	2.80 (0.31)	8.4 (0–77.4)
SEBA ($n = 24$)	2.80 (0.27)	1.1 (0–56.6)
MTA ($n = 24$)	2.85 (0.28)	100 (26.1–100)

margins (14.3%). A significantly greater percentage of specimens with underfilled margin ($P < 0.001$, chi-squared test) were observed in the MTA subgroup compared with the other two materials; while no significant differences were found in the percentage of samples with continuous ($P = 0.076$), overfilled ($P = 0.108$) and noncontinuous ($P = 0.368$) margin.

Microstructural findings

Scanning electron micrographs of the longitudinal sections revealed different structural characteristics of the root-end filling materials (Fig. 4a–c). Typical images revealed that particle size varied from coarse (IRM and sEBA) to fine (MTA). The surface of IRM was comparatively flat, uniform and appeared to be constructed of many closely packed and splinter-shaped interconnected crystals with scattered narrow voids of varied configuration on the split surface (Fig. 4a). All the samples filled with sEBA displayed a rough surface composed of sphere-like microparticles with multiple voids between them along the split surface (Fig. 4b). On the other hand, MTA samples had a more integrated and highly fused surface texture characterized by the presence of capillaries (Fig. 4c).

Discussion

Microcracks

The root apex of each tooth was sectioned with a diamond finishing bur under water cooling. Even though microcracks were not observed after root-end resection, it could not be excluded that this might have caused potential damage in the root, and disruptions could have been made during resection that became apparent during the cavity preparation procedure (Rainwater *et al.* 2000).

The occurrence of microcracks during root-end cavity preparation with ultrasonic tips has been

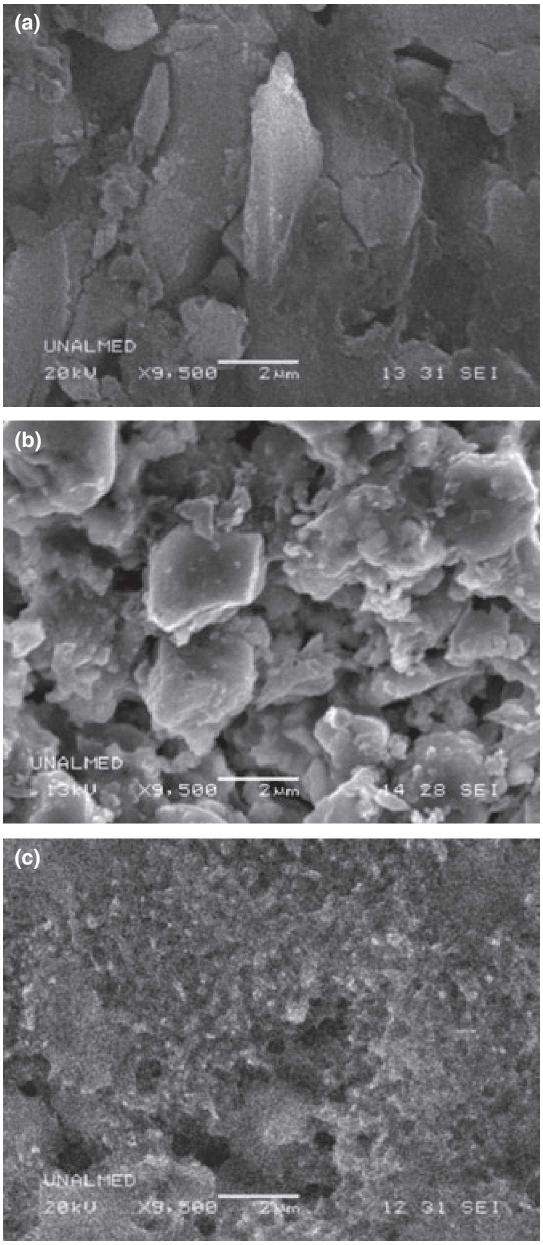

Figure 4 Scanning electron micrographs of representative polished longitudinal sections of root-end fillings. (a) Surface of IRM showing closely packed and splinter-shaped interconnected crystals with some spaces on the split surface. (b) Structure of sEBA showing a rough surface composed of fine and coarse sphere-like merged microparticles. (c) Capillary structure of MTA; a highly fused surface texture is evident. Original magnification ×9500.

thoroughly investigated (Saunders *et al.* 1994, Abedi *et al.* 1995, Frank *et al.* 1996, Lloyd *et al.* 1996, Min *et al.* 1997, Rainwater *et al.* 2000, Navarre & Steiman 2002, De Bruyne & De Moor 2005). However, only partial information is available concerning formation of microcracks following ultrasonic cavity preparation when different root filling techniques are used. In this regard, Onnink *et al.* (1994) reported that the number of cracks may not be influenced by the root filling technique. Although, in the present study, a significantly greater number of microcracks were detected in the TF group, it is not the obturation technique which influences the amount of cracks, but probably the increased time needed to remove the plastic carrier of the Thermafil system from the root canal during root-end cavity preparation. As the effectiveness of ultrasonic tips rely on thermoplasticizing the gutta-percha (Abedi *et al.* 1995), an increased preparation time in the TF group may weaken the dentinal walls. In addition, the significant correlation between the number of microcracks and the ultrasonic preparation time in both groups could explain, at least in part, the higher incidence of crack formation with ultrasonic tips observed by some authors (Abedi *et al.* 1995, Frank *et al.* 1996, Min *et al.* 1997).

On the other hand, it is generally accepted that the strength of a root-filled tooth is directly related to the amount of remaining tooth structure (Zandbiglari & Schäfer 2006). In this study, each group comprised roots with similar cross-sectional dimensions, which eliminates the dimension variation factor. Although it has been found that most cracks develop in the thinnest walls surrounding root-end cavity preparations (Abedi *et al.* 1995) or in small diameter roots (Frank *et al.* 1996), the lack of correlation between the number of microcracks and the $R_{ef} : R_{es}$ ratio in this study indicates that microcracks formation might be independent of the thickness of surrounding dentine and the cross-sectional area of the root-end cavity. These findings are in agreement with those described by De Bruyne & De Moor (2005) who found no indication of a risk of more cracks on thin dentine walls during root-end preparation.

Microleakage

Although dye penetration continues to be a method used for leakage studies, a high degree of variation in the results, which are often contradictory, has been found even when the same materials and methods were used. In this study, the dye penetration method was selected as it detected pathways of leakage not only via through-and-through voids but also by apical cul-de-sac-type voids (Taschieri *et al.* 2004). Therefore, Indian ink was chosen because its particle size is larger than other dyes (Ahlberg *et al.* 1995); some particles are small enough to enter dentinal tubules (Youngson *et al.* 1998); it remains stable during the processing of the samples (Zaia *et al.* 2002); and it allows quantitative measurement of the extent of dye penetration by linear measurements techniques (Ahlberg *et al.* 1995, Youngson *et al.* 1998, Öztan *et al.* 2001). Other advantages of using Indian ink are that it does not stain the dentine and shows the leakage pattern only (Öztan *et al.* 2001). In addition, it has been stated that bacterial ingress and Indian ink penetration provided a similar rank order for the sealing ability of the materials tested (Chong *et al.* 1995) thus detecting microleakage channels of similar size (Youngson *et al.* 1998). Notwithstanding these advantages, the *ex vivo* penetration of dye into canals should not be considered to be directly comparable with the *in vivo* leakage of irritants out of the root canal system. Instead, dye penetration should be considered as an indicator of the potential for leakage (Oliver & Abbott 2001), because a filling material that does not allow penetration of small molecules, such as dyes, has the potential to prevent leakage of larger substances, such as bacteria and their byproducts (Aqrabawi 2000).

Under the experimental conditions of this laboratory study, the ZOE-based root-end fillings of sEBA and IRM had significantly less leakage than MTA. These results are different from those reported by others (Martell & Chandler 2002, Gondim *et al.* 2005) who, using different experimental conditions, observed that MTA was significantly superior to ZOE-based root-end fillings with respect to leakage. Nevertheless, in other studies (Fogel & Peikoff 2001, Reeh & Combe 2003) MTA did not resist leakage. According to the present results, it is likely that exposure of MTA to a water-soluble dye before achieving full set contributed to the greater degree of leakage. Moreover, the dye penetration could take place in the MTA root-end filling because it is a hydrophilic aggregate material that requires moisture for its setting reaction (Yatsushiro *et al.* 1998). This may partly explain why the dye could penetrate the full length of the MTA root-end fillings. Nevertheless, it seems possible that further hydration of MTA powder by moisture can result in an increase in compressive strength and decrease in leakage (Kubo *et al.* 2005). These results, however, should be interpreted cautiously because, although in a clinical situation MTA might be exposed to blood or saline contamination before achieving full set, the evidence suggests that the ability of MTA to set properly and to maintain an adequate seal should not be affected (Montellano *et al.* 2006). Moreover, Sarkar *et al.* (2002, 2005) demonstrated that MTA has the ability to precipitate hydroxyapatite (HA) crystals in the presence of synthetic tissue fluids, such as phosphate buffered saline, which may be relevant in minimizing leakage thereafter.

Marginal adaptation

Marginal adaptation constitutes an indirect method to compare the sealability of root-end filling materials (Stabholz *et al.* 1985). The results obtained in the current study show that the root-end filling materials IRM and sEBA both gave better results than MTA in terms of uniformity of marginal adaptation. According to Fitzpatrick & Steiman (1997), the final smoothing with a finishing bur over IRM and sEBA may produce superior marginal adaptation and reduce the amount of discrepancies in the height between the root-end fillings and dentinal walls at the filling margins. Conversely, MTA cannot be subjected to final smoothing using finishing techniques because of its prolonged setting time (3 h). The present results agree with those described by Peters & Peters (2002) who described a substantial number of overfilled sEBA and underfilled MTA root-end fillings before and after occlusal loading *ex vivo*. Other authors have also reported that the outer layer of MTA dissolves in a moist environment, resulting in the loss of a surface layer of the material (Yatsushiro *et al.* 1998, Davis *et al.* 2003). However, it must be kept in mind that under *in vivo* conditions, this surface disintegration of root-end filling material might not take place to such an extent or might be self-limiting (Peters & Peters 2002). Furthermore, it has been stated that a further expansion of the material while setting in a moist environment can occur (Gondim *et al.* 2003, 2005). Based on the data presented herein, it is not possible to state which factors may prevent or limit the loss of MTA and to influence its clinical performance.

Microstructure

Microstructure of root-end filling materials has been evaluated in some investigations in which it has been observed that a degree of porosity is characteristic of dental cements prepared by mixing powder and liquids (Fridland & Rosado 2003). It is possible that there may be some artefacts in the present microstructures (i.e. volumetric shrinkage, dense/porous patches, voids, gaps and microcracks), caused during sample processing and SEM observation, that could obscure the typical surface topography of the root-end filling materials. Nevertheless, the representative images of microstructures appear to be comparable with those of the polished samples seen under stereomicroscope and do not appear to be artefacts.

IRM is a polymethyl methacrylate resin-reinforced ZOE that sets by forming a chelate between two molecules of eugenol and one molecule of zinc oxide (Craig 1997). This chelate is known to hydrolyse in the presence of water to release eugenol (Hashieh *et al.* 1998) and, according to Belli *et al.* (2001), the voids observed between zinc oxide particles are probably the result of the high vacuum used for SEM and may represent regions where eugenol evaporated. The findings in this study are consistent with those described by Yoshikawa *et al.* (1997) using the SEM who observed that the spaces in the ZOE cements did not pass completely through the sealer, allowing little or no penetration of Indian ink. Furthermore, the larger particles in IRM cement and the uniform surface between zinc oxide particles may contribute to a less microleakage and better marginal adaptation compared with MTA.

On the other hand, sEBA is an aluminium oxide-reinforced form of ZOE-based cement, which also contains *o*-ethoxybenzoic acid. It is probable that during the mechanical polishing procedure, the presence of brittle alumina particles had contributed to increase the surface roughness in these samples. Notwithstanding, despite this surface roughness, it could be considered that a micromechanical interlocking between sEBA particles and chemical bonding of the cement to dentinal walls might play an important role in microleakage resistance and good marginal adaptation noted with this material.

Finally, in contrast to ZOE cements, the split surface of MTA exhibited numerous pores and capillary structures, which could constitute an important cause of leakage. Although Torabinejad *et al.* (1995) argued that this porosity is due to the incorporation of microscopic air bubbles during the mixing operation, Fridland & Rosado (2003) observed that mixing MTA and water forms a porous matrix which retains a soluble fraction of calcium hydroxide that is able to be transferred to an aqueous environment. Thus, an important osmotic effect, which is dependent on the amount of water, may be responsible for this porosity. The fact that MTA releases calcium into these pores and capillaries, helping initiate HA crystal formation, means that these voids may fill with HA (Bozeman *et al.* 2006).

Conclusion

Microcracks following ultrasonic preparation of root-end cavities occurred independently of dentinal walls thickness and may be associated with the prolonged ultrasonic preparation time required for the removal of root fillings during root-end cavity preparation. Although sEBA and IRM had better behaviour than MTA regarding microleakage and marginal adaptation, it is possible that exposure of MTA to a water-soluble dye before achieving full set and its porous microstructure contributed to the results.

Acknowledgements

This research was supported by the Research Development Committee of the University of Antioquia (CODI-Code 0032002).

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