doi:10.1111/j.1365-2591.2007.01320.x

The effect of the canal-filled area on the bacterial leakage of oval-shaped canals

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Abstract

De-Deus G, Murad C, Paciornik S, Reis CM, Coutinho-Filho T. The effect of the canal-filled area on the bacterial leakage of oval-shaped canals. *International Endodontic Journal*, **41**, 183–190, 2008.

Aim To compare the sealing ability and the canal-filled area (gutta-percha + sealer) of three current filling techniques in oval-shaped canals.

Methodology A total of 50 oval-shaped root canals were prepared and root filled as follows: G1: lateral condensation ($n = 10$), G2: System B ($n = 10$) and G3: Thermafil system ($n = 10$). All teeth were mounted in a two-chamber apparatus and the coronal access was exposed to human saliva. The appearance of turbidity in the BHI broth over a 15-week period was observed. A cross section of each tooth was made 5 mm from the apex and the samples were prepared for microscopic analysis. Digital image measurements of the cross-sectional area and the area filled by gutta-percha and sealer were performed. Log-rank and Fisher's exact tests were used to analyse the leakage data. Student's *t*-test was used to analyse the filled-area data.

Results Overall, 30% of the specimens of G1 and G2 and 20% of G3 demonstrated leakage after 15 weeks and no significant difference was found amongst the groups ($P > 0.05$). The percentage of canal-filled area was 68, 70 and 78%, respectively ($P > 0.05$). The correlation analysis revealed no significant relation between the pattern of bacterial leakage and canal-filled area ($P = 0.128$).

Conclusions No significant difference in apical sealing and canal-filled area in oval-shaped canals was seen between the three filling techniques. No significant correlation was found between the quality of the apical seal and the filled-area of the root canal space.

Keywords: bacterial leakage, canal-filled area, filling technique.

Received 11 September 2006; accepted 2 July 2007

Introduction

An oval shape may make it difficult to clean and fill the root canal space (Wu & Wesselink 2001). Kersten *et al.* (1986) reported that both lateral condensation of gutta-percha cones and vertical compaction of warm gutta-percha were used widely in endodontic treatment, although their quality may differ in canals with different shapes. Wu *et al.* (2000a) reported a high percentage of oval-shaped canals in the apical portion of human tooth roots (≥50%) with their diameter tending to decrease apically. It could be postulated that oval-shaped canals may be filled more effectively by warm gutta-percha techniques, provided they are clean (Jacobson *et al.* 2002). Areas beyond the reach of the mechanical action of endodontic instruments in canals with oval shapes can frequently be unfilled when the lateral condensation technique is used (Wu *et al.* 2000a).

Studies have evaluated the quality of the apical seal using dyes, bacterial leakage, electrochemistry techniques and through the use of a fluid transport method. The most frequently used method is dye penetration.

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Despite its popularity, ease of use and large number of reports, dye leakage has several negative aspects that allow great variation in results from similar experiments (Wu & Wesselink 1993). The dye-leakage experimental model is subjective, less comparative and has poor reproducibility (Wu & Wesselink 1993). Studies that use a bacterial tracer derived from specific cultures or from human saliva are considered to be more reliable than tests with dye (Siqueira *et al.* 1999). This method may be considered the most adequate because it provides results more aligned to the clinical situation and especially when human saliva is used as a bacterial source. Nevertheless, the results from laboratory studies may not be directly extrapolated to the clinical situation. Bacterial leakage studies also allow the evaluation of samples at specific periods of time and preserves the sample (Siqueira *et al.* 1999). Nonetheless, it is a static model that does not simulate exactly clinical conditions, needs a long period of observation and does not allow quantification of the number of penetrating bacteria (Siqueira *et al.* 1999, 2000).

Qualitative (Siqueira *et al.* 1999) and quantitative leakage tests (Wu & Wesselink 1993) have been widely used to evaluate the quality of root fillings as well as cross sections of filled roots (Kersten *et al.* 1986, Wu & Wesselink 2001, Wu *et al.* 2002, De-Deus *et al.* 2006a,b) and the association of both (Wu *et al.* 2001, Ardila *et al.* 2003).

The aims of this study were to compare systematically the sealing ability and the canal-filled area (gutta-percha + sealer) associated with three filling techniques (Thermafil, lateral condensation and System B) in oval-shaped canals. The relationship between the canal-filled area and sealing ability was also tested.

Materials and methods

Sample selection

This study was revised and approved by the Ethics Committee, Nucleus of Collective Health Studies, Rio de Janeiro State University, Rio de Janeiro, Brazil. A total of 170 left and right mandibular incisor teeth free from cracks and with similar anatomical characteristics were selected from the tooth bank of Rio de Janeiro State University. The teeth were autoclaved and kept in 0.5% NaOCl for no longer than 7 days. Periapical radiographs were taken in buccolingual and mesiodistal directions for each tooth. Teeth with oval-shaped canals were selected only when the ratio of the long : short diameter was ≥2.5 at 5 mm from the apex (Wu *et al.* 2000b, Wu & Wesselink 2001). All teeth with isthmi, lateral, accessory or two canals were excluded. Therefore, only 57 incisors were classified as single oval-shaped canals. Seven teeth were discarded leaving a total sample of 50 teeth that were stored in 10% neutral formalin.

Instrumentation

Access to the root canal system was prepared and the patency of each canal was confirmed by inserting a size 15 file through the apical foramen before and after completion of root canal preparation. The working length was established by deducting 1 mm from the canal length. The coronal and middle third of each canal was prepared using Gates Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland), sizes 4, 3 and 2. The apical third was prepared with Flexofiles® (Dentsply Maillefer) sizes 40, 35, 30 and 25 in a balanced force technique (Roane *et al.* 1985). The canals were irrigated between each file with 1 mL of freshly prepared 5.25% NaOCl using a disposable Luer-lock syringe and 27-gauge needle. The 50 teeth received a flush of 5 mL of 17% EDTA (pH 7.7) for 3 min to remove the smear layer. After completion of the preparation a final flush of 3 mL of 5.25% NaOCl was performed. The canals were dried with paper points (Dentsply Maillefer).

The prepared teeth were randomly divided in three equal groups ($n = 10$ per group). Ten teeth with intact crowns served as negative controls and 10 teeth that were not obturated served as positive controls.

Canal filling

The canals were filled using either lateral condensation technique (G1), System B heat source (G2) (Buchanan 1996) or the Thermafil system (G3) (Johnson 1978). The teeth were filled using Grossman sealer (Endofill; Herpo Ltd, Petrópolis, RJ, Brazil). The sealer was prepared according to the instructions of the manufacturer. A size 40 file was used to pick up a measured amount of sealer (1.25 mL) from the mixing pad and placed into the canal whilst rotating it counterclockwise (Wu *et al.* 2001).

In G1, a size 25 master gutta-percha cone (Diadent Group International, Chongchong Buk Do, Korea) was placed in the canal to the full working length. Lateral compaction was achieved in each canal by using 10 accessory gutta-percha cones (MF; Diadent Group International) and endodontic finger spreader size B (Dentsply Maillefer). A heated instrument was used to remove the excess coronal gutta-percha.

In G2 the System B (model 1005; EIE/Analytic, Redmond, WA, USA) was used as recommended by the manufacturer and by Buchanan (1996). The tip of a medium-sized nonstandardized gutta-percha cone (Diadent Group International) was trimmed back until tug-back was achieved at working length. An M plugger (EIE/Analytic), which penetrated to within 6 mm of the working length was selected and a rubber stopper placed. The System B unit was preset to 200 °C during the condensation of the primary gutta-percha cone (down-pack), to 100 °C when adapting and condensing the apical portion of the secondary (backfill) gutta-percha cone, and finally to 250 °C to thermosoften the remainder of the secondary cone prior to vertical condensation.

In G3, a size 25 Thermafil verifier was used to check the size of the canal. The Thermafil Plastic Obturator (Dentsply Tulsa Dental Products, Tulsa, OK, USA) was heated in a Thermaprep Oven (Dentsply Tulsa Dental Products) for 30 s according to the manufacturer's instructions. Firm apical pressure was used to insert the Thermafil Obturator to the working length. A round diamond bur in a turbine handpiece was used to cut the plastic shaft at 1–2 mm within the access cavity; the excess gutta-percha was removed with an instrument.

The crowns of teeth were removed leaving roots 10 mm in length and the filled roots were stored at 37 °C and 100% humidity for 7 days to allow setting of the sealer.

Polymicrobial leakage

Two coats of nail varnish were applied on the external surface of all teeth, except 2 mm around the apical foramen, in on attempt to prevent bacterial leakage through lateral canals or other discontinuities in the cementum.

The apparatus used in this study was modified from that described previously by Imura *et al.* (1997) and included an hermetically sealed culture chamber (Fig. 1). All roots were inserted in eppendorf plastic tubes of 1.5 mL volume (Elkay, Shrewbury, MA, USA). The interface between the tooth/eppendorf was placed, under pressure, into the rubber stopper of a penicillin bottle, which was cut smaller, so it fitted inside the glass flask. The junctions between the root, the eppendorf and the rubber were sealed with cyanoacrylate adhesive (Loctite 496; Henkel Ltd, São Paulo, Brazil). The system was sterilized overnight using ethylene oxide gas and then placed in a 9 mL sterilized glass flask containing 4 mL of sterile Brain Heart Infusion broth (BHI; Oxoid Ltd, Basingstoke, UK), so that approximately 2 mm of the root apex was immersed in the broth. The interface was sealed with cyanoacrylate and Parafilm M (Laboratory Film; American National Can, Chicago, IL, USA).

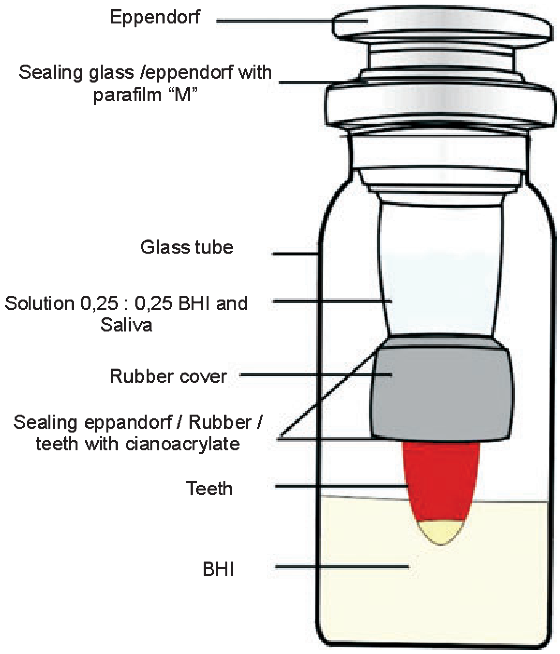

Figure 1 Setup of model design.

To verify the efficiency of the cyanoacrylate seal, 2 mL of 1% sterile methylene blue dye was placed into the tube leading to the coronal portion of each sample (Malone & Donnelly 1997). If the medium became blue, this meant the seal was defective and the specimen was discarded. The whole apparatus was incubated at 37 °C for 7 days to ensure sterilization.

The eppendorf reservoirs were filled with human saliva (20 mL) mixed in BHI broth in a 1 : 1 (v/v) ratio (Siqueira *et al.* 1999) and replenished every 3 days. Human saliva was collected from one individual at 9 am on each day of exposure or solution change. The volunteer did not brush or floss for at least 12 h before collection. Chewing a 1-g piece of Parafilm (American National Can, Menasha, WI, USA) was used to stimulate salivary flow (Gomes *et al.* 2003). The system was incubated at 37 °C and checked daily for the appearance of turbidity in the BHI broth during the following 15 weeks.

Sectioning and image analysis

After this time, each sample was sectioned horizontally 5 mm from the apical foramen using a low-speed saw (Isomet, Buhler, Ltd, Lake Bluff, NY, USA) with a diamond disc (diameter 125 × 0.35 × 12.7 mm), with continuous water irrigation in order to prevent overheating. Subsequently, each sample was embedded in an epoxy resin cylinder (Arazyn 1.0; Ara Química, SP, Brazil) to facilitate manipulation and improve the metallographic preparation. The margins adjoining the epoxy resin and tooth were sealed with cyanoacrylate (Super bonder Gel; Lockite, Itapevi, SP, Brazil).

The metallographic preparation was performed as described previously (De-Deus *et al.* 2006a,b). Specific sandpapering (DP-NETOT 4050014; Struers, Denmark) for metallographic preparation was completed. The purpose of this procedure was to obtain a surface that was free from scratches and deformities, whilst introducing as few changes as possible. To eliminate deformities from fine sandpapering and to obtain a highly reflective surface, the specimens were polished before they were examined under the microscope. The polishing was completed with diamond paste of 4–1 μm roughness (SAPUQ 40600235, Struess, Ballerup).

The samples were examined under a light optical microscope (Axiscoppe; Carl Zeiss Vision Gmbh, Hallbergmoos, Germany) and images were acquired at a 100× magnification as tagged image file images (TIFF). Image analysis and processing were completed using a CARNOY 2.0 image system for Windows (Laboratory of Plant Systematics, K.U. Leuven, Belgium). Through this software, the cross-sectional area of the canal and the filled area were measured and the percentages of filled area (gutta-percha + sealer) were calculated. The long and the short diameter of the canal cross section were measured with the aim of confirming the pre-classification of the teeth. The data obtained by a computer-assisted evaluation were repeated twice to ensure reproducibility.

Statistical analysis

Before the experiment, the sample size was calculated with a type I error set at 0.05 ($\alpha = 0.05$), a type II error set at 0.20 ($\beta = 0.80$) and an index effect size of 1 ($d = 1$).

All leakage data were organized in a contingency table. The log-rank test was used to analyse the leakage data for all groups at intervals of 6, 9, 12 and 15 weeks. Further analysis comparing the leakage pattern for the two groups demonstrating the greatest and least cumulative leakage was carried out using the Fisher's exact test (Adamo *et al.* 1999).

Student's *t*-test was used to analyse the filled-area data. A correlation analyses was also used to find if there was an association between the filled area and bacterial leakage.

A level of significance in all tests was set at $P < 0.05$. Statistical Package for Social Sciences (SPSS/PC + STATISTICS 4.0 software; SPDD International BV, Gorinchem, the Netherlands) and ORIGIN 6.0 (Microcal Software, Inc., Northampton, MA, USA) were also used as analytical tools.

Results

Leakage analysis

No growth was observed when checking the sterilization of the whole apparatus. After 3 weeks, no leakage was observed in any sample of the experimental groups. No sample in the negative control group demonstrated leakage during the 15-week experiment. On the other hand, all specimens of the positive control group showed broth turbidity within the 3rd day of incubation.

At 6 weeks, only one out of 10 samples (10%) in group 1 (lateral condensation) demonstrated leakage.

At 9 weeks, one out of 10 samples (10%) in G2 (System B) and one out of 10 samples (10%) in G3 (Thermafil) demonstrated leakage.

At 12 weeks, one sample (10%) of G1, two samples (20%) of G2 and one sample (10%) of G3 demonstrated leakage.

At 15 weeks, a total of three samples (30%) in G1 and two samples (20%) of G2 demonstrated leakage. Two samples (20%) in G3 also demonstrated leakage. None of the negative controls had demonstrated leakage.

Statistics

Average leakage time for all groups was 69.5 days. The comparison of the leakage pattern using the Fisher's exact test demonstrated no significance differences amongst the three groups ($P > 0.05$). These results are plotted in Fig. 2.

Checking the canal shape

The canal shape was checked by measuring the shorter and longer axis of the canal. A pre-established radiographic proportion was defined in order to select oval-shaped root canals, hence the ratio of the long : short diameter was ≥0.4 (1/2.5) (Wu *et al.* 2000b, De-Deus *et al.* 2006b). The ratio of the long : short diameter in the root canals had a mean value of 0.62. The difference between radiographic and actual canal cross-sectional ratios must be a consequence of root canal instrumentation. Overall, the effectiveness of the radiographic method used to classify the root canal shapes was confirmed although the final ratio following canal preparation was different.

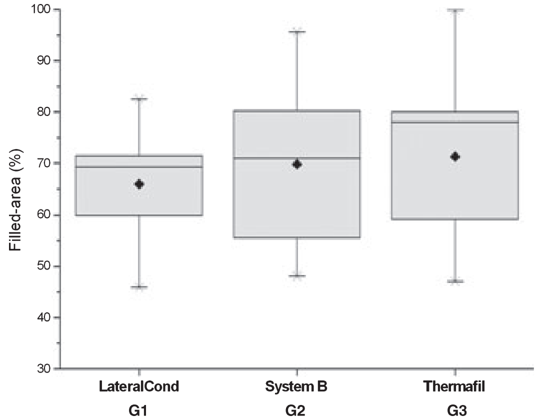

Figure 3 Pattern of filled-area in each group.

Canal-filled area

In G1, the filled area varied from 45.88 to 82.61%, with a mean of 68.15 (10.98%). G2 had a filled area varying from 48.08 to 95.69%, with a mean of 70.15% (16.96%). The filled area of G3 varied from 47.02 to 98.9%, with a mean of 78.31% (15.82%). These data are expressed in Fig. 3. The relationship between the cross-sectional area and the filled area is described in Fig. 4. Figures 5–7 illustrate the filled area in each group. No significant difference was found between the lateral condensation, system B and thermafil groups ($P > 0.05$).

Correlation analysis of the leakage and filled-area data

No significant correlation was found between the leakage and the filled area of the root canal space in each group ($P = 0.128$).

Discussion

The present study was designed to compare the sealing ability and to quantify gutta-percha and sealer used in the same samples. The results demonstrated that at the end of 15 weeks, the leakage

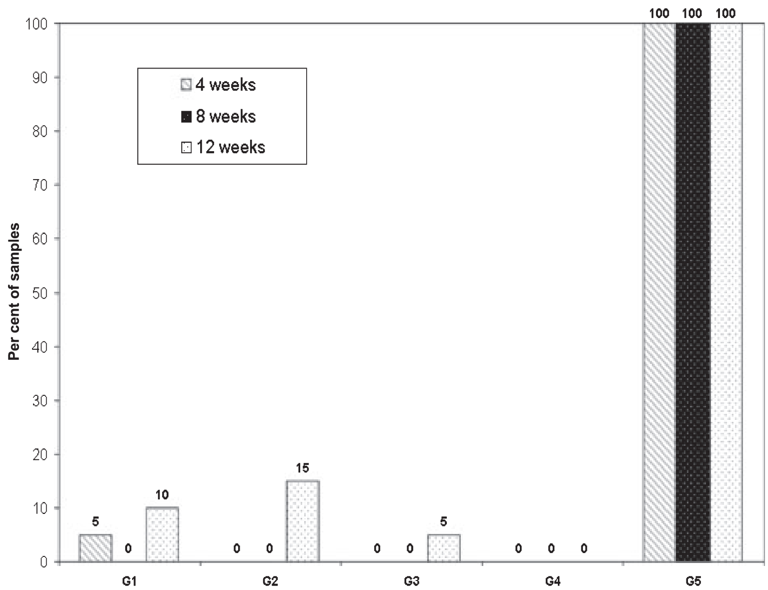

Figure 2 Percentage of samples in each group with leakage over a period of 15 weeks.

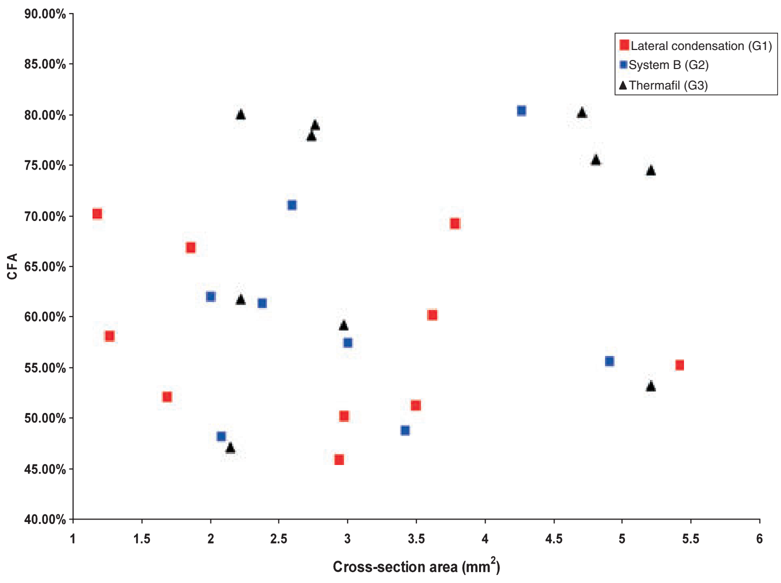

Figure 4 Relationship between the cross-sectional area and the filled-area in each group. CFA: canal filled area.

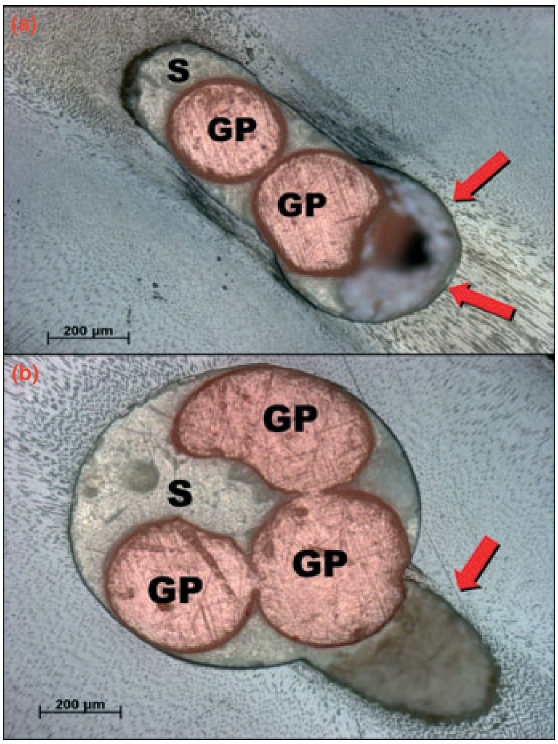

Figure 5 Representative cross sections of two specimens (a and b) from G1. Red arrows: void areas; GP: gutta-percha; S: sealer.

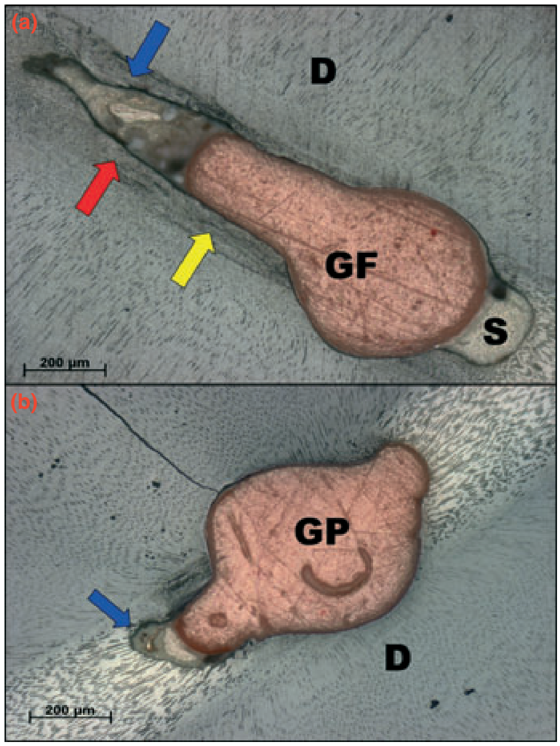

Figure 6 Representative cross sections of two specimens (a and b) from G2. Red arrows: void areas; blue arrow: sealer; yellow arrow: close adaptation between sealer and gutta-percha; D: dentine; GP: gutta-percha; S: sealer.

was minimal in all groups. Ricucci & Bergenholtz (2003) reported that well-prepared and filled canals resisted bacterial penetration even when directly exposed to the oral environment. Siqueira *et al.* (2000) in a bacterial leakage study, demonstrated that there was no statistical difference between

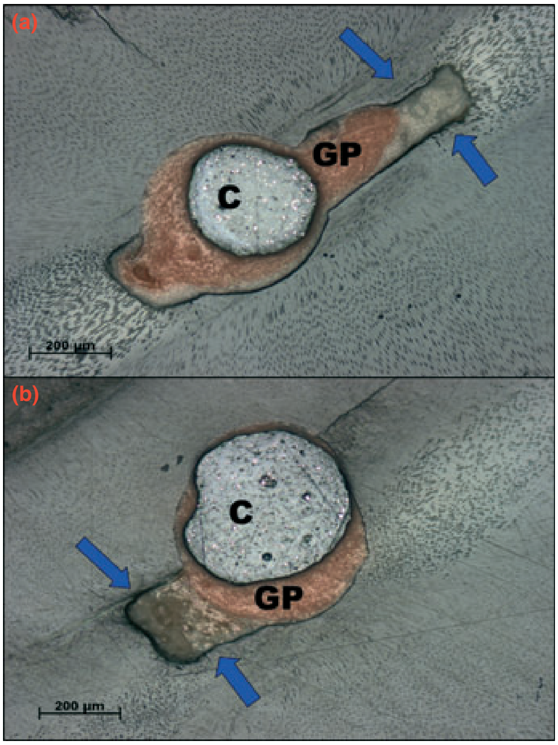

Figure 7 Representative cross sections of two specimens (a and b) from G3. Blue arrows: sealer; Red arrows: void areas; C: Thermafil carrier; GP: gutta-percha.

continuous wave of condensation, thermafil and lateral condensation, but there was a high percentage of contamination of the samples. Juhász *et al.* (2006) reported that root canal form influenced short-term sealing ability.

The results of the present study agree with several leakage studies, which evaluated the quality of different root fillings techniques (Siqueira *et al.* 2000, Wu *et al.* 2001, Jacobson *et al.* 2002, De-Deus *et al.* 2006b). On the other hand, the results are at variance to most studies in relation to bacterial penetration in filled teeth, without coronal seal.

Under the experimental design of the present study, the Thermafil system samples produced the highest canal-filled area (CFA) (78.31%). Nevertheless, no significant statistical difference was found between the experimental groups. Generally speaking, the canal-filled area revealed voids in the oval-shaped root canals. These findings contradict those described by De-Deus *et al.* (2006a), where the Thermafil system produced higher percentages of gutta-percha-filled areas. In agreement with De-Deus *et al.* (2006a), Gençoglu *et al.* (2002) reported that Thermafil system produced significantly greater percentage of gutta-percha-filled area than lateral condensation and System B techniques. These differences can be explained by methodological variations.

De-Deus *et al.* (2006a) measured only the percentage of gutta-percha-filled area whereas, in the present study, the entire filled area (gutta-percha + sealer) was measured.

One of the factors that might influence the different performance of techniques may be related to the anatomy of the canals. Oval-shaped canals are difficult to clean and fill (Wu *et al.* 2000b, 2001). Moreover, van der Sluis *et al.* (2005) reported that the poorer results of root fillings in oval canals could be explained because it is not possible to completely instrument the entire circumference with use of the balanced force technique; when the canal wall and the recesses are not clean it is then not possible to fill the canal completely.

It may be postulated that irregularly shaped canals may be filled more effectively by warm gutta-percha techniques, provided they are clean (Wu *et al.* 2001). Areas beyond the reach of the mechanical action of endodontic instruments in canals with irregular shapes can frequently remain unfilled when the lateral condensation technique is used (Wu *et al.* 2001). These findings do not corroborate with the results of the present study in which the lateral condensation technique, System B as well as the Thermafil system performed similarly, and showed a good sealing ability. van der Sluis *et al.* (2005) also reported no correlation between fluid transport leakage and the percentage of gutta-percha-filled area. These authors provide an explanation for this poor relationship and it can be applied for the current results, that is 'the percentage of gutta-percha-filled area may give a poor image of the root canal filling at the level of the section, but the void detected may be of the *cul de sac* type and not run from the coronal to the apical therefore not showing fluid transportation'.

The current leakage results reassert the findings reported in a previous study where the sealing ability in oval-shaped canals was also investigated (De-Deus *et al.* 2006b). Wu *et al.* (2000a) reported that there was no difference in leakage between lateral condensation and warm vertical condensation; however, the CFA was greater using warm gutta-percha than using cold gutta-percha technique in oval-shaped canals. The leakage patterns, as well as the filling ability of each technique were statistically similar.

Conclusion

Under the conditions of the present *ex vivo* evaluation the following conclusions can be drawn: (i) both apical sealing and canal-filled area in oval-shaped canals were statistically similar in the three techniques tested, (ii) no significant correlation was found between the quality of the apical seal and the filled area of the root canal space and (iii) the quality of root canal filling in oval-shaped canals is compromised.

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