A preliminary analysis of the morphology of lateral canals after root canal filling using a tooth-clearing technique

M. Venturi¹, C. Prati², G. Capelli³, M. Falconiº & L. Breschi³
¹Private Practice, Bologna, ²Dipartimento di Scienze Odontostomatologiche, University of Bologna, Bologna, ³Dipartimento di Scienza e Società, University of Cassino, Cassino, ºDipartimento di SAU e FAL, University of Bologna, Bologna, Italy

Abstract


Aim This study used a modified tooth-clearing technique to allow observation of accessory canals following filling with a warm gutta-percha technique and one of two endodontic cements.

Methodology Ten extracted human maxillary molars with three roots were selected and divided into two equal groups of five teeth. Each group had 15 canals. Root canal preparation was performed with a modified double flared technique; irrigation with 5% NaOCl and lubrication with RC-Prep were used. The canals were then filled with gutta-percha and cement utilizing a warm vertical condensation technique in the apical third followed by thermo-mechanical compaction in the middle and coronal thirds. Pulp Canal Sealer or AH-Plus were used in the experimental groups. The teeth were demineralized with a modified buffered acid solution, cleared in methylsalicylate and examined under a stereomicroscope. Accessory canals were evaluated in the apical, middle and coronal thirds of each root canal and categorized as narrow or wide, following observation on four surfaces. The depth of penetration of gutta-percha and cement into lateral canals was scored using a 5-point system.

Results Complete transparency of the roots was achieved. Accessory canals were detected in all specimens. In coronal ramifications, gutta-percha filled the empty spaces (coronal thirds, grades 3 and 4: 70.9% in AH-Plus group and 68.8% in Pulp Canal Sealer group). In the apical accessory canals, gutta-percha entered less frequently (apical thirds, grades 3 and 4: 17.9% in the AH-Plus group and 3.2% in the Pulp Canal Sealer group); cement without gutta-percha (grades 1 and 2) was present in 55.5% in the AH-Plus group and 38.7% of the Pulp Canal Sealer group. Analysis showed that AH-Plus cement resulted in significantly greater filling of the apical accessory canals compared to Pulp Canal Sealer.

Conclusions The modified tooth-clearing technique allowed observation of fine morphological details in all specimens. Effective gutta-percha filling was evident in most of the wide coronal lateral canals whilst the apical narrow ramifications were often incompletely filled by cement. Overall AH-Plus demonstrated better diffusion into lateral accessory canals compared to Pulp Canal Sealer.

Keywords: accessory canals, demineralization, endodontic cements, gutta-percha, root canal, tooth clearing.

Received 28 January 2002; accepted 5 September 2002

Introduction

It has been recommended that root filling should provide a complete, three-dimensional filling of the main root canal and of all accessory canals, in order to prevent the leakage of fluids and microorganisms (Schilder 1967). Although O’Neill et al. (1983) found no correlation between the microscopic appearance of gutta-percha adaptation in cleared teeth and the degree of apical leakage, other studies have demonstrated endodontic failure due to patent accessory canals (Nichols 1963, Seltzer et al. 1967) and success after filling lateral...
canals (Genyuan & Zhongni 1984, Weine 1984). Warm vertical condensation of gutta-percha has been considered to be effective at filling the ramifications of the root canal system (Schilder 1967, Dulac et al. 1999, Silver et al. 1999).

Previous studies have compared different techniques for their ability to fill the root canal system (O’Neill et al. 1983, Reader et al. 1993, Pallares & Faus 1995, Gulabivala et al. 1998, De Moor & Martens 1999, Dulac et al. 1999, Kytridou et al. 1999, Silver et al. 1999). Some studies used natural roots with simulated lateral canals or resin blocks with simulated main and accessory canals (Reader et al. 1993, Dulac et al. 1999, Silver et al. 1999) in an attempt to create similar experimental groups. For example, using this approach, it is possible to have the same accessory canal diameter and angle in relation to the main canal. However, if artificial resin blocks, it is difficult to create narrow or curved accessory canals.


Some studies showed a better performance of resin-based endodontic cements in relation to sealing than non-resin-based ones (Taylor et al. 1997, Haïkel et al. 1999, De Almeida et al. 2000); however, the relationship between the flow of sealer and its ability to penetrate narrow accessory canals has not been investigated. It has been established that the flow of a root canal sealer is important as it reflects its capacity to penetrate into small irregularities and also into lateral canals (Grossman 1976). If the material flows well, it will not completely obliterate all extensions of the root canal system (Benatti et al. 1978). Limkangwalmongkol et al. (1991) reported that AH-26 appeared to have many advantages over other sealers as it mixed easily and flowed well. AH-26 has been subsequently replaced by AH-Plus that, according to the manufacturer, has the same physical properties of AH-26 but no longer releases formaldehyde. These resin-based cements are two-paste materials whereas many zinc oxide–eugenol-based sealers are powder and liquid formulation. It has been reported that standards for correct proportioning of sealer components are nonexistent and that the individual prepares the material to a consistency of choice (Benatti et al. 1978). As a result, their flow may change in relation to the mixing procedure. Moreover, it has been demonstrated that there is a relationship between the age of eugenol and flow, i.e. as eugenol ages, sealer flow increases (Mendonça et al. 2000).


Various techniques have been used to clear teeth and a number of demineralizing agents have been proposed: 5–11% nitric acid (Kasahara et al. 1990, Saunders & Saunders 1992, Tagger et al. 1994, Berutti 1996, Ibarrola et al. 1997); 20% formic acid (O’Neill et al. 1983); 40% solution of ion exchange resin and formic acid (Felton et al. 1991) or 5% hydrochloric acid (Vertucci 1978). It has been reported that shrinkage of organic tooth tissue could occur during the demineralization process, and that this phenomenon may be avoided if a weak concentration of acid is used (Robertson et al. 1980).

The aims of this investigation were to evaluate: (i) the ability of a modified buffered acid tooth clearing technique to allow observation of fine morphological details in order to evaluate root canal fillings; (ii) the quality of accessory canal filling utilizing vertical condensation of warm gutta-percha in the apical third of the canal, followed by thermo-mechanical compaction of gutta-percha in the middle and coronal areas; (iii) the ability of two endodontic cements (AH-Plus and Pulp Canal Sealer) to fill accessory canals.

Materials and methods

Preparation of teeth

Ten noncarious extracted human molars made up of maxillary eight first molars and two second molars were selected. A radiograph of each tooth was exposed. None of the teeth had restorations, roots with resorption, fractures or open apices. Calculus and debris on the root surface was removed using size 7/8 Gracey curettes (Hu-Friedy Mfg. Co., Chicago, IL, USA). Diameters of the
apical foramina were measured directly on the tooth using a stereomicroscope (Zeiss Stemi 2000-C, Carl Zeiss Jena GmbH, Zeiss Group, Jena, Germany). The teeth were divided in two groups on the basis of the foramina size, each made up of four maxillary first molars and one maxillary second molar, giving a total of 15 canals. Specimens were prepared by the same operator under 3.5× magnification (Designs for Vision, Ronkonkoma, NY, USA). Conventional endodontic access was achieved using a tapered diamond bur (Number 845.314.012, Komet Brasseler, Lemgo, Germany) mounted on a contra-angle hand piece (Kavo Intramatic 25C, Kavo GmbH & Co., Biberach, Germany).

**Canal instrumentation**

Working lengths were established by introducing a size 06 K-file until the tip could be seen at the foramen under 3.5× magnification (Designs for Vision). Canal instrumentation was performed with a modified double flared technique, using stainless steel K-files size 06–40 (F.K.G. Dentaire, La Chaux-de-Fonds, Switzerland) and size 1 and 2 Gates–Glidden burs (Dentsply-Maillefer, Ballaigues, Switzerland).

The root canals were first enlarged in the coronal region using size 1 and 2 Gates–Glidden burs. Apical preparation began with the first K-file to bind the canal at the working length and continued through sequentially larger K-files. Following the use of the size 20 or 25 K-file at working length, the apical and middle region were cleaned and shaped using K-files with a step-back preparation. Enlargement of the foramen diameter was avoided and apical patency was maintained. Final taper in all canals was achieved with the next larger K-file than that used in apical preparation and consisted of working progressively with three or four larger K-type files to length 1 mm short of the previous instrument length.

Lubrication was provided by RC-Prep (Hawe Neos Dental, Bioggio, Switzerland) placed on each instrument and irrigation was done with 5% NaOCl. In each canal, the total volume of NaOCl was 10 mL. Irrigation was performed after each instrument during the initial stages of canal enlargement, whilst NaOCl was used only after every recapitulation during the final stages. Following preparation, each canal was left full of hypochlorite so that the total time of irrigation was 1 h.

Each canal was dried with paper points and a cotton pellet was fixed with wax on the external surface of each root to cover the foramina. The specimens were then fixed to a grid and immersed in a thermostatic water bath at 37 °C so that the access cavities were above the water surface. Specimens of group A were filled with MF gutta-percha cones (Kerr Co., Romulus, MI, USA) and AH-Plus cement (Dentsply DeTrey GmbH, Konstanz, Germany); specimens of group B were filled with MF gutta-percha cones and Pulp Canal Sealer (Kerr Co.). Prior to their insertion, the MF gutta-percha cones were inserted and adjusted until they reached working length. Each cone gave tugback in the apical region and then it was shortened by 1 mm. Cement was introduced using a size 15 K-file and the cone was then positioned.

Warm vertical condensation was performed following a conventional procedure (Schilder 1967) using a Touch’n Heat Device (Analytic Technology, Redmond, WA, USA). The tip of the instrument was held at a distance of 5 mm from the apex and pluggers (Hu-Friedy Mfg. Co.) were used in the coronal and middle thirds. In the apical region, condensation was performed by finger pluggers size 30 (Kerr Co.), 3 mm from the apex. After apical compaction, a gutta-percha cone was inserted in the canal, the coronal end was severed with the Touch’n Heat and the middle and coronal thirds were filled by thermomechanical condensation using Gutta-Condensor size 30 (Dentsply Maillefer). The instrument was slowly pushed apically whilst rotating to 5 mm from the apex and then retracted during rotation with a push and pull movement and adapted further by hand condensation.

**Clearing technique**

The roots were sectioned from the crowns, the wax and pellets removed and the specimens immersed for 14 days in the following demineralizing solution: 7% formic acid; 3% hydrochloric acid; 8% sodium citrate in aqueous solution. The solution was changed every 3 days and the specimens were kept under continuous agitation (Agitator 722, Asal srl, Milan, Italy). Specimens were rinsed in running tap water for 2 h, immersed in 99% acetic acid overnight, rinsed in distilled water, dehydrated in ascending concentrations of ethanol at 25, 50, 70, 90, 95 and 100% (30 min passage each), and finally cleared and stored in methyl salicylate.

**Morphological and statistical analysis**

Morphological analysis was performed using a stereomicroscope (Zeiss Stemi 2000-C, Carl Zeiss Jena GmbH) to reveal details of accessory canal filling. The microscope was used with a graded lens and measurements were taken utilizing a micrometer. Observations were
performed by two independent observers who counted the number of visible lateral canals within the coronal, middle and apical third of the roots.

In the present study, the accessory canals were categorized as wide when the diameter of opening in the main canal was over 80 μm, and narrow when it was under 40 μm. This measure was taken on each of the accessory canals.

All accessory canals were observed on four surfaces (mesial, buccal, distal and palatal), with increasing magnifications from 5 to 40×. Every space detected between the canal wall and the surface of the canal filling was recorded. Five scores were defined in order to evaluate the filling of accessory canals:

- **No filling (grade 0):** Filled with cement only <10% of their total length.
- **Partial filling with cement without gutta-percha (grade 1):** Filled with cement not up to their total length, or not three-dimensionally, thus empty spaces were identifiable.
- **Complete filling with cement without gutta-percha (grade 2):** Filled three-dimensionally and up to their total length by cement, without presence of gutta-percha, or with gutta-percha up to 50% of their total length.
- **Full filling with cement and partial filling with gutta-percha (grade 3):** Filled three-dimensionally up to their total length by cement in which gutta-percha penetrated from 50 to 90% of their total length. Nevertheless, the space not filled by gutta-percha was completely filled by cement.
- **Full filling with cement and gutta-percha (grade 4):** Totally filled with cement and gutta-percha.

A micrometer was used to better identify the five different scores on the specimens. Data were analysed by means of a logistic regression analysis performed with STATA 7.0 (STATA Corporation, College Station, TX, USA).

### Results

In each group, the diameters of the foramena were 0.20 mm in six canals, 0.25 mm in six canals, 0.30 mm in two canals and 0.35 mm in one canal. The first molars had mesio-buccal root length (measured from the corresponding cusp) from 19 to 20.5 mm, disto-buccal root length from 18 to 19.5 mm and palatal root length from 20 to 22 mm. The second molars had fused roots with converging root canals, and root length between 19.5 and 20 mm. The curvature of the canals were similar: the palatal and disto-buccal roots of the first molars had essentially straight canals, whilst the mesio-buccal roots of the first molars and the roots of the second molars had curvatures from 20 to 30 degrees.

Fine morphological details could be observed in all the specimens as complete transparency was obtained (Figs 1–9). Accessory canals were identified in all the specimens in the coronal (55 in group A and 48 in group B), middle (41 in group A and 46 in group B) and apical (56 in group A and 62 in group B) thirds of the canals (Table 1).

In general, all grades of filling were found in all the different specimens. Grades 0 (Fig. 1) and 1 (Fig. 2) were considered not acceptable, whilst grades 2 (Fig. 3), 3 (Fig. 4) and 4 (Fig. 5) were considered acceptable. Scores 3, 4 or 5 were attributed only on the basis of the total absence of space under 20× vision on all four surfaces.

#### Apical third

Grade 0 (Fig. 1) was observed in 26.8 and 58.1% of the AH-Plus and the Pulp Canal Sealer group, respectively. Grade 1 (Figs 2, 4, 6 and 7) was observed in 5.3% in the AH-Plus group and in 29.0% of the Pulp Canal Sealer group. Grade 2 (Figs 3, 8 and 9) was observed in 50.0 and 9.7% of the AH-Plus and the Pulp Canal Sealer group, respectively. Grade 3 and 4 were observed in a small number of lateral canals: they occurred in 17.9% of the AH-Plus group and in 3.2% of the Pulp Canal Sealer group.

![Figure 1 Stereomicroscope image of an apical delta with four empty accessory canals (grade 0). The filling materials (Pulp Canal Sealer and gutta-percha) entered a negligible tract of two canals (wide arrows), whilst no filling can be detected in the others (+). Original magnification 25×.](image-url)
Figure 2  Image revealing three accessory canals (arrows) covered by only cement (Pulp Canal Sealer; grade 1). Cement mainly fills the most coronal and widest ramification and is less represented in the most apical and narrowest. The limit between the apical vertically condensed and the thermo-mechanically compressed gutta-percha can be seen (wide arrow). Original magnification 15×.

Figure 3  Stereomicroscope images showing middle and apical tracts of a complex endodontic system fully injected by cement (AH-Plus; grade 2). Some minor communications (arrow) between the main canals and some filled accessory canals can be seen. It should be noted that the materials seem to fill the canals even if the main canals irregularly enlarge their diameters in the apical tract. Original magnification 8×.

Figure 4  Accessory canals can be seen in the image: only the most coronal one (arrow) has been filled with gutta-percha and cement (Pulp Canal Sealer; grade 3), whilst the other narrower ones have only been filled by cement. Original magnification 15×.

Figure 5  Two accessory canals filled with gutta-percha (grade 4) on a coronal tract can be observed. Some traces induced by the waves of thermo-mechanical condensation are visible (arrows). The tested cement was Pulp Canal Sealer. Original magnification 40×.
Middle third

Grade 0 was observed in 14.6% in the AH-Plus group and in 32.6% of the Pulp Canal Sealer group. Grade 1 (Fig. 9) was observed in 31.8% of the AH-Plus group and in 26.1% of the Pulp Canal Sealer group. Grade 2 was observed in 14.6% (AH-Plus group) and 2.2% (Pulp Canal Sealer group), whilst Grade 3 (Fig. 4) and 4 (Fig. 5) together reached 39.2% (AH-Plus group) and 39.1% (Pulp Canal Sealer group).

Coronal third

Grade 0 was observed only in 3.6% of the AH-Plus group and in 10.4% of the Pulp Canal Sealer group. Grade 1

Figure 6  Image revealing two apical ramifications without complete filling. In particular, gutta-percha entered only their access (arrows), whilst the endodontic cement (Pulp Canal Sealer) sprayed the walls. Original magnification 10×.

Figure 7  A narrow (arrow) and a wider (wide arrow) accessory canal can be seen. Gutta-percha is not detectable and only an incomplete filling with cement (Pulp Canal Sealer) is visible. Original magnification 10×.

Figure 8  Image revealing a complex narrow ramifications filled by cement (AH-Plus). The homogeneous distribution of the cement inside the endodontic system should be noted. Original magnification 6×.

Figure 9  Stereomicroscope image of some narrow accessory canals injected by cement (AH-Plus). Original magnification 8×.
was observed in 16.4% of the AH-Plus group and in 18.7% of the Pulp Canal Sealer group. Grade 2 (Fig. 8) was observed in 9.1% (AH-Plus group) and 2.2% (Pulp Canal Sealer group), whilst grade 3 and 4 were often observed in the coronal accessory lateral canals (70.9% for AH-Plus group and 68.8% for Pulp Canal Sealer group). Most of these lateral ramifications were approximately 40–100 μm wide.

Table 2 shows the ‘not acceptable’ and ‘acceptable’ filling of lateral canals. Statistical analysis revealed that regardless of the position of the lateral canals, group A specimens (with AH-Plus cement) had significantly higher filling scores ($P < 0.05$) than Group B specimens (with Pulp Canal Sealer cement; Table 2). Overall, a higher rate ($P < 0.05$) of acceptable filling (grades 2–4) was achieved in the coronal level, compared to the apical level.

A major difference was found at apical level between the cements where specimens of group A revealed a threefold greater number of canals compared to specimens of group B ($P < 0.05$). Middle and coronal third lateral canals revealed minor differences between group A and B, confirming a higher penetration rate for AH-Plus specimens.

### Discussion

#### Clearing technique

The tooth-clearing technique is a useful method for obtaining information on root canal systems and for evaluating root fillings. A variety of techniques to demineralize and clear teeth have been investigated previously: 5–11% nitric acid (Kasahara et al. 1990, Saunders & Saunders 1992, Tagger et al. 1994, Berutti 1996, Ibarrola et al. 1997); 20% formic acid (O’Neill et al. 1983); 40% solution of ion exchange resin and formic acid (Felton et al. 1991); or 5% hydrochloric acid (Vertucci 1978). The most common procedures use aggressive demineralizing solutions, i.e. 5–11% nitric acid solutions (Robertson et al. 1980, Tagger et al. 1994), with the aim of reducing the time of demineralization (Kasahara et al. 1990, Saunders & Saunders 1992, Ibarrola et al. 1997). The technique is simple to perform, rapid and gives good results in few days, compared to the longer application (more than 2 weeks) required with other methods (Hasselgren & Tronstad 1975). The demineralization process may be enhanced by using higher concentrations of acid solution or by raising the temperature, but in both cases it is important to achieve a good contact between the tooth and the acid solution.
cases this might result in excessive demineralization; shrinkage and damage of the organic component may also occur (Robertson et al. 1980, Kwan & Harrington 1981).

The use of weak acid solutions allows better control of shrinkage of the organic tooth structure (Robertson et al. 1980). The modified tooth-clearing technique proposed in this study was performed with a weak acid solution characterized by the presence of sodium as a buffering agent. The additional immersion in acetic acid improved the quality of the dentine matrix due to its capability to fix organic components. Excellent and homogeneous decalcification was also achieved by continuously agitating the acid solution and by changing it every 3 days. The reduced aggressiveness of the acid procedure took more time for specimen preparation, but allowed excellent control of the technique resulting in good and useful transparency that demonstrated the morphology of apical and lateral canals.

The final step of the procedure used methyl salicylate, which tolerates more water and is less noxious than other clearing agents such as xylene (Robertson et al. 1980). Methyl salicylate is also less toxic than other agents (Okumura 1927, Seeling & Gillis 1973, Vertucci et al. 1974).

Accessory canal filling

In the present study, a greater number of accessory canals was detected compared to other reports (Manning 1990). This may be owing to the fact that stereomicroscope observations were performed after root canal filling, thus permitting visualization of narrow ramifications.

Several studies have evaluated the ability of filling techniques to effectively penetrate and adapt to the canal walls under different in vitro conditions. Accessory canals with diameters of 0.1 mm or more have been drilled in natural teeth (Smith et al. 2000), or resin blocks (Dulac et al. 1999, Silver et al. 1999). In the present study, the filling of natural accessory canals was evaluated: very narrow ramifications were examined and the relationship between their diameters and location in the different regions of the roots was recorded. Overall, gutta-percha demonstrated a better ability to penetrate wide accessory canals located in coronal and middle thirds than smaller apical ones (Schilder 1967, Dulac et al. 1999, Silver et al. 1999). Three major parameters may be involved in determining these results: (i) the viscosity of gutta-percha that fills large spaces compared to a narrow one; (ii) the thermo-mechanical compaction of gutta-percha (performed in the middle and coronal thirds) that developed high compaction forces due to the presence of the condensed filling of the apical third; (iii) further compaction of the middle and coronal thirds of the gutta-percha performed by pluggers. This allowed protracted compaction of the gutta-percha (in the middle and coronal thirds) that was simultaneously plasticized and pushed against the solid apical gutta-percha without risk of apical extrusion. In particular, the high compaction forces are directed towards gutta-percha of the apical third and are then diverted against the lateral walls, forcing the gutta-percha into accessory canals and resulting in a complete three-dimensional filling of the ramifications of the middle and coronal regions. The thermo-mechanical compaction also mixed gutta-percha and cement so that a homogenous and well-adapted root canal filling could be observed in the canals (O’Neill et al. 1983).

The presence of cement filling in the narrow ramifications of the apical third canals is in agreement with previous studies (Schilder 1967, Dulac et al. 1999, Silver et al. 1999). This is probably related to the simultaneous lack of softening and ineffective compaction of the gutta-percha. In fact, it has been demonstrated that warm vertical condensation techniques usually produce small temperature rises in gutta-percha close to the apex (Schilder et al. 1985, Marciano & Michailesco 1989, Sweatman et al. 2001). Smith et al. (2000) found that the deeper the heat was applied, the better was the adaptation achieved. However, it must be stressed that softening of gutta-percha in the apical region may lead to poor control of the material, with possible extrusion (Schilder 1967, ElDeeb 1985). Silver et al. (1999) suggested that the quality of gutta-percha adaptation is compromised in the apical root canal if heat is applied 2 mm from the apex.

The presence of small amounts of cement on the internal walls, without producing a true three-dimensional filling, was frequently observed. This finding means that in most occasions the radiographic observation of full apical accessory canals may be incorrect. In fact, the cements are radiopaque materials and the penetration of a thin layer of the material in such narrow ramifications could create the illusion of a true three-dimensional filling. Although these radiographic findings may lead to misleading interpretations in relation to the filling of lateral accessory canals, the positive aspect of the partial penetration of gutta-percha and/or cement, according to Schilder (1967), is related to a well-compacted gutta-percha and cement, with a strong and well-distributed pressure, usually producing a true three-dimensional filling in the main root canal.
The morphological analysis revealed excellent penetration of AH-Plus endodontic cement particularly into narrow apical lateral canals. In fact, in apical regions, complete filling of the accessory canals with cement (without gutta-percha) was observed especially in the AH-Plus group, whilst the Pulp Canal Sealer group demonstrated a poor penetration. These findings might clarify previous data that showed a better performance of AH-Plus in relation to sealing than other non resin-based endodontic cements (Haikel et al. 1999, De Almeida et al. 2000). The better diffusion of AH-Plus can be enhanced by heating as it becomes more fluid, suggesting that it can be particularly appropriate for warm obturation techniques.

Conclusions

A buffered acid demineralizing solution made it possible to preserve the morphological characteristics of the roots and to obtain good transparency.

The root canal filling technique used in this study, with a gutta-percha warm vertical condensation followed by a thermo-mechanical compaction, filled the most coronal wide accessory canals with gutta-percha and cement, whilst the apical narrow canals were incompletely filled by cement.

AH-Plus demonstrated better diffusion and a greater ability to penetrate narrow and deep spaces than Pulp Canal Sealer.

References


