doi:10.1111/j.1365-2591.2007.01307.x

Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and *in vivo*

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Abstract

Mamootil K, Messer HH. Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and *in vivo*. *International Endodontic Journal*, **40**, 873–881, 2007.

Aim To compare the depth and consistency of penetration of three different root canal sealer cements into dentinal tubules in extracted teeth and to measure the penetration of an epoxy resin-based sealer cement *in vivo*.

Methodology Root canals of 50 extracted human pre-molar teeth were prepared and obturated using three different sealer cements based on epoxy resin (AH26), zinc oxide eugenol (Pulp Canal Sealer EWT) and methacrylate resin (EndoREZ). Five teeth filled without sealer were used as controls. Teeth were sectioned and prepared for observation using scanning electron microscopy. A further 12 teeth with a history of successful root filling and subsequent extraction were collected and sectioned. The depth of sealer penetration into dentinal tubules was measured and the consistency and appearance of the sealer within the tubules observed.

Results AH26 demonstrated the deepest penetration (1337 μm), followed by EndoREZ (863 μm) and Pulp Canal Sealer EWT (71 μm). The difference in penetration between all sealer groups was found to be statistically significant ($P < 0.05$). The resin-based sealers appeared to penetrate tubules more consistently. In the clinical cases, all teeth demonstrated sealer penetration to varying depths (98–1490 μm).

Conclusions The depth and consistency of dentinal tubule penetration of sealer cements appears to be influenced by the chemical and physical characteristics of the materials. Resin-based sealers displayed deeper and more consistent penetration. Penetration depths observed for the epoxy resin-based sealer *in vivo* were consistent with that found in the experimental model.

Keywords: AH26, EndoREZ, Pulp canal sealer EWT, sealer cements, tubule penetration.

Received 16 May 2005; accepted 17 May 2007

Introduction

The main objectives of root canal treatment are the elimination of microorganisms from the root canal space and the prevention of reinfection. Chemomechanical preparation is considered the most important step in the management of the infected root canal system; however, it is difficult or even impossible to eliminate completely all organisms from the canal space (Peters *et al.* 2002). Bacteria can persist in areas such as lateral canals and dentinal tubules, as these areas may provide protection from the disinfecting actions of irrigants and medicaments (Ørstavik & Haapasalo 1990). These remaining bacteria may play a role in persistent periapical disease (Oguntebi 1994).

A number of studies have shown that most teeth with apical periodontitis will heal despite having a positive bacterial culture at the time of root filling (Sjögren *et al.* 1997, Peters & Wesselink 2002). Filling may be able to overcome some of the limitations of chemomechanical preparation with the main aim being firstly to eliminate all avenues of leakage from the oral cavity and the periradicular tissues into the root canal system by creating a fluid tight seal (Gutmann & Witherspoon 2002); and secondly to eliminate space and seal within the root canal system any irritants that cannot be fully removed during

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cleaning and shaping the procedures (Sjögren *et al.* 1997). This concept of bacterial entombment suggests that bacteria remaining within the root canal space are rendered harmless as they are deprived of essential nutrients and space required for growth and proliferation.

The use of a sealer cement in conjunction with a core filling material is recommended with most obturating techniques (Gutmann & Witherspoon 2002). Gutta-percha (GP) has no bonding properties to dentine regardless of the filling technique employed (Evans & Simon 1986). Sealer cements create a union between the core material and the canal wall by filling any residual spaces (Hata *et al.* 1992). In addition, sealer cements often have the ability to penetrate areas such as lateral canals and dentinal tubules. The penetration of sealer cements into dentinal tubules is considered to be a desirable outcome for a number of reasons: it will increase the interface between material and dentine thus improving the sealing ability and retention of the material may be improved by mechanical locking. Sealer cements within dentinal tubules may also entomb any residual bacteria within the tubules and the chemical components of sealer cements may exert an antibacterial effect that will be enhanced by closer approximation to the bacteria (Heling & Chandler 1996).

Penetration of sealer cements into dentinal tubules is influenced by a number of factors including smear layer removal, dentine permeability and filling technique (White *et al.* 1984, 1987, Okşan *et al.* 1993, Kouvas *et al.* 1998, De Deus *et al.* 2004). Variations in the physical and chemical properties of sealer cements also influence the depth of penetration (Okşan *et al.* 1993). The ability of any one particular sealer cement to penetrate dentinal tubules consistently and effectively will be one of many factors influencing the choice of material for filling. It is therefore important to compare the penetrability of different types of cements used. It is also important to validate the results from *in vitro* studies with findings from clinical cases. To date, only one study has reported sealer penetration of dentinal tubules *in vivo* (Vassiliadis *et al.* 1994).

The aims of this study were to compare the depth and consistency of penetration of three different root canal sealer cements into dentinal tubules in extracted teeth, and to investigate the penetration and appearance of an epoxy resin-based sealer cement into dentinal tubules when placed *in vivo*. The sealers selected for the laboratory study were an epoxy resin-based sealer (AH26; De Trey, Dentsply, Konstanz, Germany), a zinc oxide eugenol (ZnOE)-based sealer (Pulp Canal sealer EWT; Kerr, Sybron Dental Specialties, Romulus, MI, USA) and a methacrylate-based resin sealer (EndoREZ; Ultradent Products, South Jordan, UT, USA).

Materials and methods

Part A: comparison of sealer cements in extracted teeth

Fifty extracted intact single-rooted human pre-molar teeth were selected for the experimental study and stored in 10% buffered formalin. After conventional endodontic access cavities were prepared, the teeth were checked for the presence of a single root canal. Once selected, the teeth were stored in isotonic saline. A size 10 K-file was introduced into each canal until it could be seen through the apical foramen and the length measured. Working length was established by subtracting 0.5 mm from this length.

The canals were prepared by a single operator using rotary nickel-titanium ProTaper® and ProFile® endodontic instruments (Dentsply Maillefer, Ballaigues, Switzerland). The ProTaper S1 and S2 files were used to the working length to prepare the coronal and middle thirds of each canal. After coronal flaring, a K-file was used to gauge the apical size. The apical third was then prepared using successive 0.04 taper ProFiles until a master apical rotary (MAR) size of 45 or 60 was achieved depending on the size of the first K file to bind at the working length.

Each canal was irrigated with 1% sodium hypochlorite (NaOCl) solution during preparation. 1 mL of NaOCl was used between successive files. Apical patency was maintained by passing a size 10 K-file through the apical foramen between files. After completion of canal preparation, the canals were irrigated with 5 mL 15% liquid ethylenediaminetetracetic acid (EDTA, Colgate Oral Care Company, Waverly, Australia) and 5 mL NaOCl to remove the smear layer. Each solution was left in the canal for approximately 3 min. A final rinse of 5 mL distilled water was used to remove any remnant of the irrigating solutions. The canals were dried using paper points. The teeth were kept moist at all times by wrapping them in saline-soaked gauze. After preparation, five teeth were randomly selected as controls. The remaining 45 teeth were randomly divided into three groups for filling using three different sealer cements.

Group 1
AH26 (De Trey, Dentsply, Konstanz, Germany) sealer cement was mixed according to manufacturer's instructions at a powder : liquid ratio of 2 : 1 on a glass slab at room temperature. Sealer was placed into the canals using a size 25 Lentulo spiral (Dentsply Maillefer, Ballaigues, Switzerland) in a slow speed handpiece introduced to approximately 2 mm short of the working length.

Group 2
Pulp Canal Sealer EWT (Kerr, Sybron Dental Specialties) was mixed according to manufacturer's instructions on a glass slab. Sealer was placed into the canals using a size 25 Lentulo spiral in a slow speed handpiece introduced to approximately 2 mm short of the working length.

Group 3
EndoREZ (Ultradent Products Inc, South Jordan, UT, USA), a two-part chemical set material was mixed in an automix nozzle. According to the manufacturer's recommendations, the material was dispensed into a narrow diameter syringe (Skini^{TM} syringe) with a fine tipped cannula (NaviTip^{TM}). The NaviTip^{TM} was inserted into the canal 2 to 3 mm short of the working length and the sealer was dispensed whilst withdrawing the syringe. Sealer was placed into the canal until the level of the sealer reached just short of the canal orifice.

All canals were filled using 0.04 tapered master GP cones matched to the final MAR file used to prepare the canal. The master cone was fitted into the canal prior to the placement of the sealer cement and checked for 'tugback' at working length. After sealer cement placement for the experimental groups, the master cone was seated to working length in a slow plunging motion. The remaining canal space was filled by lateral compaction of nonstandardized accessory GP cones. For the control group, no sealer cement was used. Excess GP was removed from the access cavity using a hot instrument and a coronal seal of Cavit (ESPE, Dental AG Norristown, PA, USA) placed. The teeth were then stored at 37 °C and 100% humidity for 14 days to allow the sealer cements to set.

Part B: teeth filled *in vivo*

Twelve teeth with a history of root filling and subsequent extraction were obtained from private endodontic practices. The teeth had been extracted because of periodontal disease, unrestorable caries or vertical root fracture. Teeth extracted because of endodontic post-treatment periapical disease were excluded. Where extraction was required because of root fracture, the fractured root was excluded from analysis. The root canals had been prepared either with hand instruments or rotary NiTi endodontic instruments. NaOCl and EDTA were known to be used as irrigants in all cases. All canals were filled with laterally compacted GP and AH26 as the sealer cement.

Specimen preparation

All teeth were sectioned at 5 and 7 mm from the anatomical root apex using a 0.3 mm thick diamond blade (Struers, Rødovre, Copenhagen, Denmark) at slow speed with constant water-cooling. The cuts were made perpendicular to the long axis of the root thereby obtaining a 2 mm thick specimen. The surface representing the 5 mm level was selected as the surface to be analysed and this surface was demineralized with a 10 min application of 15% EDTA. A further 10 min application of 5% NaOCl was used to remove any organic debris and the surface layer of organic matrix around the sealer tags. The sections were then washed with distilled water and dried using a gentle stream of air. The sections were mounted onto aluminium stubs using an adhesive carbon tab and placed into a dry heat oven at 50 °C for 48 h for complete dehydration. The sections were then sputter coated with a thin gold coating prior to observation using a scanning electron microscope (SEM). The maximum depth of sealer penetration was measured for each section using a calibrated measuring tool, which was incorporated into the microscope control system.

Data analysis

The mean and median values for maximum sealer penetration of each of the experimental groups were calculated along with 95% CI. Statistical analysis was performed by using the Mann–Whitney test to compare the penetration of each sealer. Significance was set at the 5% level ($P < 0.05$).

Results

Experimental groups

Scanning electron microscope observation of the control teeth confirmed the absence of material within

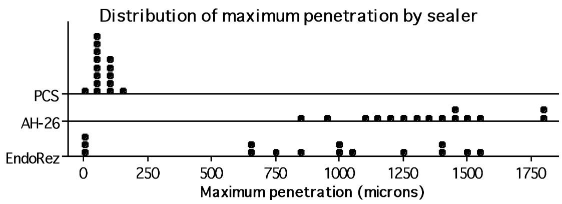

Figure 1 Dot plot of distribution of maximum penetration by sealer. PCS, pulp canal sealer.

the dentinal tubules in this group. One tooth in the Pulp Canal Sealer EWT group and three in the EndoREZ group had no sealer penetration. The distribution of maximum penetration by sealer is presented in Fig. 1. There is a clear difference between the mean penetrations of the ZnOE-based sealer and the resin-based sealers. The differences between AH26 (1337 μm) and Pulp Canal Sealer EWT (71 μm) and between EndoREZ (863 μm) and Pulp Canal Sealer EWT were highly significant ($P < 0.005$). The comparison of penetration depth between AH26 and EndoREZ was also found to be significantly different ($P = 0.01$).

The consistency of tubule penetration varied amongst the resin-based sealers and the ZnOE-based sealer. Resin-based sealers were observed to penetrate most of the patent tubules present (Figs 2 and 3). The ZnOE-based sealer penetrated the patent tubules less frequently (Fig. 4). The appearance of the sealers within the tubules also differed amongst the sealer types. The ZnOE-based sealer was granular in appearance especially at the peripheral side away from the canal wall. The resin-based sealers were observed as long smooth walled rods completely filling the tubule

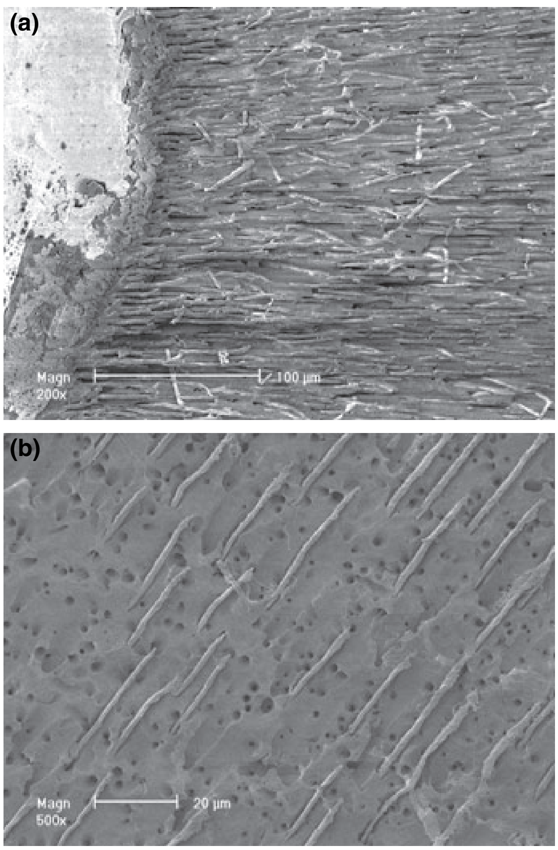

Figure 2 (a) Penetration of epoxy resin-based sealer (AH26) at the canal-dentine border. Extensive resin tags are seen penetrating deep into the dentinal tubules. (b) Higher magnification of epoxy resin tags in the outer dentine. The sealer appears as intact rods completely filling the tubule space.

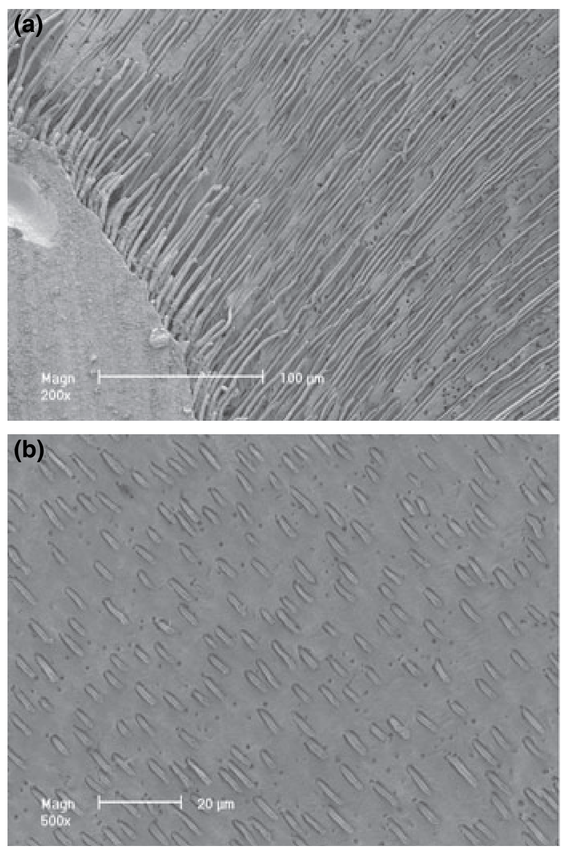

Figure 3 (a) Penetration of methacrylate resin-based sealer (EndoREZ) at the canal-dentine border. Extensive penetration into dentine is observed. The sealer appears as long smooth walled rods. (b) Higher magnification of methacrylate resin tags in the outer dentine.

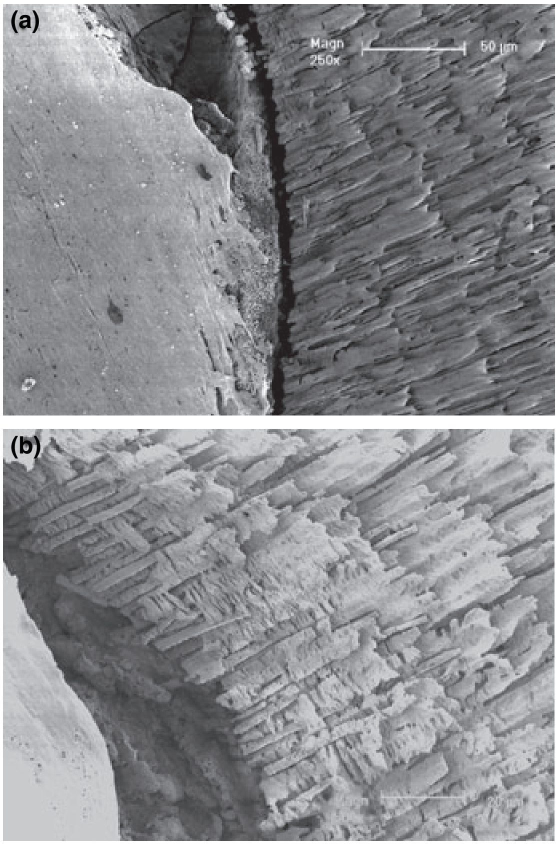

Figure 4 (a) Penetration of zinc-oxide eugenol-based sealer (Pulp Canal Sealer EWT) at the canal-dentine border. Consistent tubule penetration is not observed. (b) Higher magnification of zinc-oxide eugenol-based sealer reveals short sealer tags with a granular appearance.

space (Figs 2 and 3). At higher magnification, both AH26 and EndoREZ were seen to penetrate and remain intact in the fine lateral branches between the tubules (Fig. 6).

Clinical cases

The tooth type and age of the patient at the time of filling as well as the maximum depth of penetration for each tooth are presented in Table 1. Penetration of epoxy resin sealer into dentinal tubules was observed in all of the teeth filled clinically. There was a large variation in the penetration depths observed (98–1490 μm). The resin-based sealer was observed as intact rods with smooth walls within the tubules. In those teeth with deeper penetration, sealer was seen consistently in most tubules (Fig. 5).

Table 1 Maximum penetration depth values for teeth obturated *in vivo*

Case number	Tooth type	Age	Penetration depth (μm)
1	Mandibular molar	49	1272
2	Maxillary pre-molar	53	234
3	Maxillary pre-molar	38	201
4	Mandibular molar	43	340
5	Mandibular molar	53	1458
6	Mandibular molar	52	1180
7	Mandibular molar	41	1490
8	Maxillary pre-molar	59	354
9	Maxillary canine	48	169
10	Mandibular molar	51	833
11	Maxillary molar	58	255
12	Maxillary molar	39	98

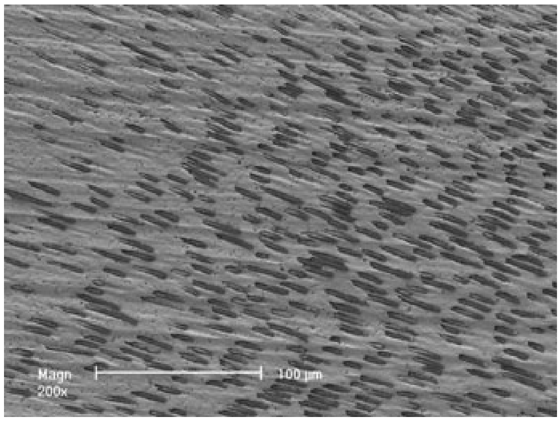

Figure 5 Epoxy resin tags in the outer dentine of a tooth obturated *in vivo*. Consistent penetration of the tubules is observed.

Discussion

Scanning electron microscopy has been used by a number of investigators to evaluate the penetration of sealer cements into dentinal tubules (White *et al.* 1984, 1987, Okşan *et al.* 1993, Vassiliadis *et al.* 1994, Kouvas *et al.* 1998, Çalt & Serper 1999, Kokkas *et al.* 2004). This particular technique offers a number of advantages. The image produced using SEM allows for highly detailed observation of the dentinal tubules and the integrity and surface appearance of the sealer cement. The adaptation of the sealer cement to the tubule can be seen in detail and at high magnification (Fig. 6). It allows for the observation of sealer cement within the tubules at distant sites from the canal wall where the density of the tubules is less. It also allows for

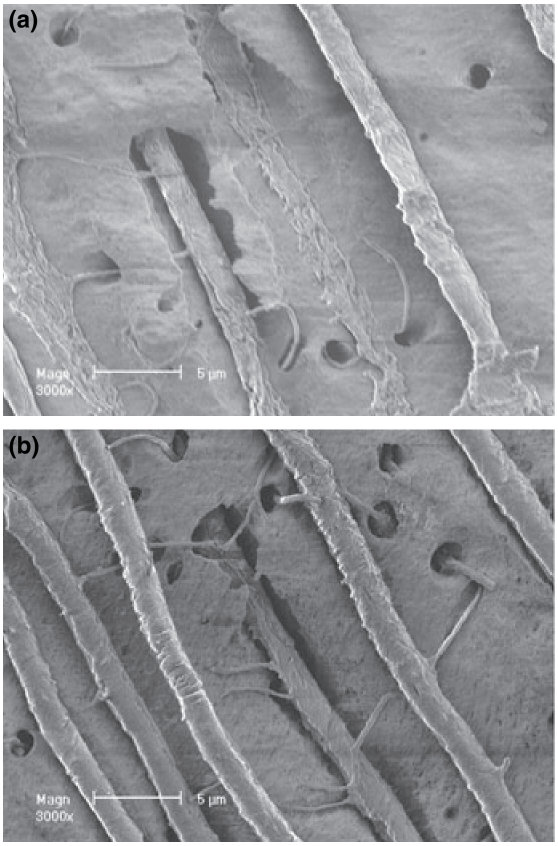

Figure 6 (a) High magnification of epoxy resin sealer close to the canal-dentine border. Penetration of sealer into the fine lateral branches connecting the tubules is observed. (b) High magnification of methacrylate resin sealer also shows penetration into lateral branches connecting the tubules.

accurate measurement of penetration depths. The main disadvantage of this technique is the inability to obtain a detailed overall view at low magnification. This makes systematic analysis more difficult. The other disadvantage of this technique is the potential for producing artefacts during the preparation of the samples for analysis.

Alternative techniques using light microscopy have also been used to analyse sealer penetration (De Deus *et al.* 2004, Weis *et al.* 2004). The major difficulty with this technique is the ability to distinguish the sealer from the dentine. De Deus *et al.* (2004) used light optical microscopy and analysis with an image processing system to distinguish the sealer from the dentine. Weis *et al.* (2004) incorporated a histological stain into an epoxy resin sealer to contrast the sealer from the dentine. A limitation of this technique is the difficulty of incorporating the dye into some sealer types and the potential for such stains to interfere with the physical properties, handling characteristics and setting times of the sealer cements. The advantage of this technique is that it allows a more systematic approach to analysis.

The use of EDTA and NaOCl to demineralize the surface layer of dentine and expose the contents of the dentinal tubules has been previously described (Gwinnett 1977, White *et al.* 1987). White *et al.* (1987) used a 20 min EDTA rinse followed by NaOCl to expose the sealer tags without disrupting them. Preliminary investigations revealed that extended exposure to EDTA resulted in excessive demineralization; therefore, a 10 min application was employed. The use of buffered formalin as a storage medium did not effect the ability of NaOCl to remove the organic tissue (Thé 1979).

Regional variation in the depth of tubular penetration has been demonstrated by a number of authors (Şen *et al.* 1996, Çalt & Serper 1999, De Deus *et al.* 2004, Weis *et al.* 2004). Deepest penetration of sealer cement has been demonstrated in the middle third of the root canal. Weis *et al.* (2004) noted significantly deeper penetration of an epoxy resin sealer into tubules 5 mm from the anatomical apex compared with 3 and 1 mm. Apical dentine displays less tubule density with some areas completely devoid of tubules (Carrigan *et al.* 1984, Mjör *et al.* 2001). The effectiveness of smear layer removal techniques is also reduced closer to the apex (O'Connell *et al.* 2000). The sections analysed in this study are representative of dentine from the middle third of the root.

The depth of penetration of the ZnOE-based sealer Pulp Canal Sealer EWT reported in this study was consistent with that reported in previous studies involving ZnOE-based sealers (<100 µm: Kouvas *et al.* 1998, De Deus *et al.* 2004, Kokkas *et al.* 2004). The reported penetration depths of resin-based sealer cements have shown greater variation. Şen *et al.* (1996) reported penetration depths of the resin-based sealer Diaket of 751–1000 µm. The authors in that study flushed the canals with 70% alcohol prior to obturation. Kokkas *et al.* (2004), on the other hand, reported the mean maximum penetration depth of AH Plus to be 54.6 µm. In these studies, longitudinal sectioning techniques were employed. The disadvantage of this orientation is that it does not allow for complete observation of all of the dentine surrounding the canal and there is potential to miss areas of deep penetration. Weis *et al.* (2004) analysing transverse sections reported that sealer penetration was the deepest and the most consistent in the buccal and lingual directions. They reported sealer penetration into the outer third of the dentine wall in these areas. This is consistent with the penetration depth reported in the present study, which also used transverse sections. To date, only one study has reported on the penetration of a methacrylate-based cement used as a root canal sealer (Ahlberg & Tay 1998). Whilst precise measurements were not given, the resins tags were reported to extend 'far into the dentinal tubules'.

The results of the present study confirm the findings of other studies that suggest that the penetration of sealer cements may be a function of their chemical and physical characteristics (Okşan *et al.* 1993, Şen *et al.* 1995). The greater penetration shown by resin-based sealers is consistent with the findings of Şen *et al.* (1996) and Okşan *et al.* (1993), who noted that the resin-based sealer Diaket had deeper penetration than other sealers used in their studies. Kokkas *et al.* (2004) also found that the epoxy resin sealer AH Plus displayed deeper penetration than the ZnOE-based sealer Roth 811. White *et al.* (1987), on the other hand, found that AH26 and Roth 811 were equally capable of penetrating dentinal tubules, although AH26 because of its greater integrity was more likely to remain intact within the tubules.

Other factors such as filling technique have been shown to be significant in the penetration of ZnOE-based sealers (De Deus *et al.* 2004), but not for the epoxy resin-based AH26 (Weis *et al.* 2004). This suggests that tubule penetration of resin-based sealers is not dependent on the hydraulic forces created during filling; rather the sealer is drawn into the tubules by capillary action. This may explain why AH26 with a longer setting time of 24–36 h exhibited significantly deeper penetration than EndoREZ with an initial setting time of 7–8 min and a hard setting time of 20–30 min.

The penetration of sealer cements into dentinal tubules is considered to be potentially beneficial to the filling. Sealer penetration increases the interface between the material and the dentine, which, in turn, may improve the mechanical retention of the material by mechanically locking it into place; this potentially reduces leakage. Two studies have attempted to relate sealer penetration to microleakage (Şen *et al.* 1996, Stevens 2003). Şen *et al.* (1996) reported that sealers which penetrated deeper into dentine tubules, displayed less microleakage compared to those with little penetration, although this relationship was not statistically significant. Stevens (2003) found that a final rinse with 95% ethanol significantly increased the depth of penetration of a ZnOE-based sealer and significantly decreased microleakage. However, the decrease in leakage could not be statistically correlated with increased sealer penetration.

The influence of the smear layer on microleakage has also been investigated, with results suggesting that smear layer removal resulted in less coronal leakage (Saunders & Saunders 1992). The influence of smear layer removal on the penetration of sealer cements has been investigated by a number of authors (White *et al.* 1984, Okşan *et al.* 1993, Kouvas *et al.* 1998, Kokkas *et al.* 2004) with general agreement that smear layer removal results in deeper and more consistent sealer penetration. Therefore, it is suggested that the decreased microleakage associated with smear layer removal may be attributable to the penetration of sealer into dentinal tubules. The smear layer removal technique employed in this study using 15% EDTA and 1% NaOCl allowed penetration of all sealer cements used.

The other main advantage of sealer penetration into dentinal tubules is the potential for these materials to exert antibacterial effects against bacteria that may reside within these areas. The potential for bacteria to colonize dentinal tubules is well established (Oguntebi 1994, Love 1996). Sealers that display greater penetration will potentially have a greater propensity to entomb viable bacteria within tubules, isolating them from potential nutrient sources. In addition, tubules occluded by sealer cements provide fewer opportunities for bacterial colonization. The chemical components of sealers have also been shown to exert antibacterial effects within the confines of the dentinal tubule (Heling & Chandler 1996).

To date, only one study has investigated sealer penetration into dentinal tubules *in vivo* (Vassiliadis *et al.* 1994). They found penetration of a ZnOE-based sealer to a depth of 200 μm in most cases despite the fact that smear layer removal techniques were not used. Two cases displayed penetration 900 μm from the canal wall, although these appeared to be incidental findings. In the present study, consistent penetration to depths exceeding 1000 μm was noted (Fig. 5). This confirms the ability of AH26 to penetrate to these depths in the clinical situation and therefore confirms the validity of the experimental model used. The variation in penetration depths noted in the clinical cases is most likely because of the variation in the tooth type observed and the relative ages of the patients at the time of filling.

Conclusions

The depth and consistency of root canal sealer penetration into dentinal tubules appears to be influenced by the chemical and physical properties of the material. The epoxy resin-based sealer AH26 and the methacrylate resin-based sealer EndoREZ displayed deeper and more consistent penetration compared with the ZnOE-based sealer Pulp Canal Sealer EWT. The depth of penetration observed for the epoxy resin-based sealer AH26 in the experimental model was consistent with the depth of penetration observed in clinically root filled canals.

Acknowledgements

This study was supported by a grant from the School of Dental Science, University of Melbourne, Australia. We would like to thank Associate Professor Ian Gordon, from the Statistical Consulting Centre, The University of Melbourne, for assisting with the statistical analysis.

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