International Endodontic Journal

doi:10.1111/j.1365-2591.2010.01806.x

Novel methodology to evaluate the effect of residual moisture on epoxy resin sealer/dentine interface: a pilot study

S. G. Gibby, Y. Wong, J. C. Kulild, K. B. Williams, X. Yao & M. P. Walker

School of Dentistry, University of Missouri-Kansas City, Kansas City, MO, USA

Abstract

Gibby SG, Wong Y, Kulild JC, Williams KB, Yao X, Walker MP. Novel methodology to evaluate the effect of residual moisture on epoxy resin sealer/dentine interface: a pilot study. *International Endodontic Journal*, **44**, 236–244, 2011.

Aim To evaluate the sealer/dentine interface associated with an epoxy resin sealer using the combination of Goldner's trichrome stain (GTS) and scanning electron microscopy (SEM) to verify the use of the experimental methodology.

Methodology Extracted human maxillary incisors (6) were subjected to root canal treatment. Subsequent to pulp removal, canal instrumentation and smear layer removal using EDTA and NaOCl, teeth were randomly and equally assigned to a 'wet' or 'dry' group. The 'dry' group was desiccated (95% ethanol/suction/paper points/air-drying), whilst the 'wet' group was treated with a saline rinse/suction/single paper point. Canals were then filled with an epoxy-based resin sealer and warm vertical gutta-percha compaction. After 7-day storage at 37 °C, roots from each group were sectioned into apical, middle and coronal horizontal subsections that were cut and split into paired halves and evaluated with GTS or SEM. With GTS sections, hybrid layer and sealer tubular penetration were measured ($n = 15$ measurements/intracanal location/condition) and evaluated using a two-factor repeated measures analysis of variance. The SEM qualitative analysis of paired sections was included as a complementary confirmation of GTS analyses.

Results In dry and wet groups, there was no conspicuous sealer/dentine interface hybrid layer, irrespective of canal location. However, dry specimens exhibited more uniform sealer distribution with deeper tubular penetration in the coronal and middle third ($P < 0.05$). In contrast, there was decreased sealer distribution and tubule penetration in the apical third, regardless of moisture condition ($P < 0.05$).

Conclusions The experimental methodology (combination of GTS and SEM) can be used to evaluate the intracanal resin sealer/dentine interface. The pilot data indicated that thorough drying of the root canal system may result in improved epoxy resin sealer distribution and deeper resin sealer tubular penetration, especially in the coronal and middle thirds of root canals.

Keywords: Goldner's trichrome stain, residual moisture, resin-based sealer, scanning electron microscopy, sealer/dentin interface quality.

Received 1 January 2010; accepted 11 September 2010

Introduction

Resin-based endodontic sealers, based on adhesive resin systems in restorative dentistry, have been available for many years. Numerous microleakage studies compared resin-based sealers to other endodontic sealers (Zmener *et al.* 1997, Roggendorf *et al.* 2007, Zmener *et al.* 2008), but relatively few studies have specifically examined the resin sealer/dentine interface (Tay *et al.* 2005, Gharib *et al.* 2007).

Whilst sealer tubular penetration is an important factor related to decreased microleakage (White *et al.* 1984, Sen *et al.* 1996), with resin-based sealers, another important factor may be sealer infiltration of

Correspondence: Dr Mary P. Walker, Assistant Dean for Research and Graduate Programs, University of Missouri-Kansas City, School of Dentistry, 650 E 25th Street, Kansas City, MO 64108, USA (Tel.: +1 816 235 2825; fax: +1 816 235 2254; e-mail: walkermp@umkc.edu).

intertubular demineralized dentine forming a hybrid layer. With the resin/dentine interface for restorative dentistry adhesives, if demineralized dentine resin infiltration is incomplete, fluid movement between dentine and hybrid layer can potentially lead to adhesive resin–dentine bond degradation(Schwartz 2006). Dentine residual moisture is critical for adequate demineralized dentine resin infiltration (Carvalho *et al.* 1996, Van Meerbeek *et al.* 2003) by preventing collagen network collapse, effectively impeding resin infiltration.

The evidence from restorative adhesive systems suggests that for optimal infiltration of a resin-based sealer into the intraradicular demineralized collagen network, the canal should not be over-dried (Carvalho *et al.* 1996, Van Meerbeek *et al.* 2003). Despite this, some clinicians deliberately desiccate the root canal system prior to sealer application, using alcohol as a final rinse to aid in the drying of canals. Whilst some studies examined intracanal moisture effect on the root canal seal (Kuhre & Kessler 1993, Horning & Kessler 1995, Roggendorf *et al.* 2007, Zmener *et al.* 2008), only two studies (Roggendorf *et al.* 2007, Zmener *et al.* 2008) included resin-based sealers but neither specifically evaluated the resin sealer/dentine interface. This interface is important, however, given that the weak link in certain resin-based sealer studies has been along the sealer/dentine interface (Tay *et al.* 2005, Doyle *et al.* 2006).

To date, there has been no reported investigation of residual moisture effect on the resin sealer/dentine interface, as defined by sealer infiltration of intertubular collagen and dentinal tubules. Whilst the quality of the coronal resin/dentine interface with restorative adhesives has been evaluated using complementary techniques such as Goldner's trichrome stain (GTS) and scanning electron microscopy (SEM) (Wang & Spencer 2004, Wang *et al.* 2006), these techniques have not been used to evaluate the intracanal resin sealer/dentine interface. The aim of this pilot study was to verify the use of the experimental methodology (combination of GTS and SEM analyses) to evaluate how residual moisture as well as intracanal location (coronal vs. middle vs. apical) affects the quality of an epoxy-based endodontic sealer/dentine interface.

Materials and methods

Preparation of teeth

Six extracted human maxillary incisors (IRB exempt collection protocol, 07–41) were de-coronated using a water-cooled, low-speed diamond saw (Buehler Ltd., Lake Bluff, IL, USA). All subsequent instrumentation and obturation procedures were performed in an environmental chamber at 33 ± 2 °C and 20 ± 2% humidity, simulating clinical conditions with a rubber dam (Plasmans *et al.* 1994).

After verifying apical patency, root canals were instrumented to a uniform size 60.04 taper (Series 29 NiTi rotary instruments; Tulsa Dental, Johnson City, TN, USA) using 1 mL of 5.25% NaOCl after each file. Following instrumentation, the smear layer was removed by irrigating with 5 mL 5.25% NaOCl for 1 min and 5 mL 17% EDTA for 1 min, an irrigation protocol established previously (Goldman *et al.* 1982, Yamada *et al.* 1983, Calt & Serper 2002).

After NaOCl and EDTA treatment, the teeth were randomly and equally assigned to two groups, 'wet' and 'dry'. The root canals of the dry group teeth were flushed with 2 mL 90% ethanol, the remaining liquid removed using a capillary suction tip (Ultradent Products, Inc., South Jordan, UT, USA) and canals dried until at least five paper points no longer displayed moisture. The canals were then air-dried for 30 s at 8 psi using a capillary tip. The canals in the wet group teeth were flushed with 2 mL saline and the remaining liquid removed using a fine suction tip, followed by placement and immediate removal of one paper point.

Size 50.04 taper gutta-percha cones (Tulsa Dental) adjusted to 60 tip size (Tip Snip gauge; SybronEndo, Orange, CA, USA) were seated to the working length. An epoxy-based resin sealer, AH Plus (Tulsa Dental), was mixed according to manufacturer's instructions. The master gutta-percha cone was lightly coated with sealer and inserted into the canal by moving the cone in a circular manner and pumping gently up and down three times to adequately coat canal walls with sealer.

All the root canals were obturated with the continuous wave of condensation technique (System-B Heat Source; SybronEndo) and backfilled with a Calamus Flow Obturation Delivery System (Tulsa Dental). After sealing the coronal 3 mm (Cavit; VWR International, West Chester, PA, USA), the teeth were stored at 37 °C in 10 mL PBS in sterilized, individual 20-mL glass vials for 7 days to allow adequate time for the sealer to set.

Teeth from each group were then sectioned horizontally into 1-mm-thick apical, middle and coronal subsections using a water-cooled diamond blade. One subsection was randomly selected from each root third (Fig. 1a). The subsections were then sectioned

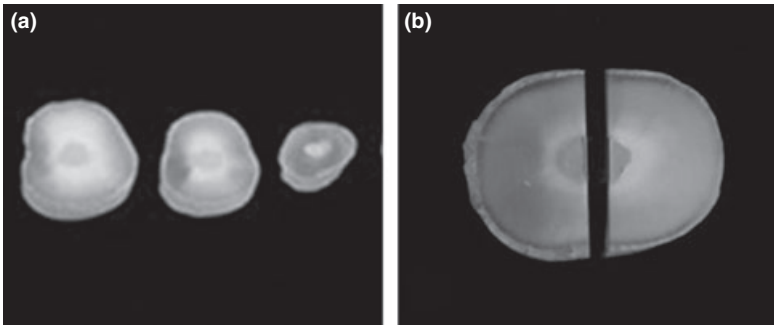

Figure 1 (a) Coronal, middle and apical root sections. (b) Matched-half subsections used for either Goldner's trichrome stain or scanning electron microscopy analysis.

longitudinally (Fig. 1b) into buccal and lingual halves with one-half evaluated using GTS and the other by scanning electron microscopy.

Goldner's trichrome stain analysis

For the GTS analysis, 8-μm-thick sections were cut from the canal subsections using a microtome tungsten carbide knife (LabX, Midland, Ontario, Canada). The microtome sections were collected on glass microscope slides treated with Haupt's adhesive, a mixture of gelatin, glycerine and phenol. Following staining, the sections were dehydrated in ascending ethanol and xylene, coverslipped with mounting media and examined at 100× using a light microscope (Nikon Inc., Melville, NY, USA). With GTS, exposed protein stains a distinct red, the mineral stains green, and the colour of resin/resin-coated protein can vary depending on the chemistry of the resin (Spencer & Swafford 1999, Spencer *et al.* 2000, Walker *et al.* 2002). Photomicrographs of five randomly selected microtome sections from each canal location were evaluated using an imaging programme (Analysis; Soft Imaging System Corp, Lakewood, CO, USA) with the intention of qualitatively evaluating the intracanal sealer distribution and quantitatively measuring the hybrid layer width in addition to the width of any exposed subjacent protein. Linear measurements of resin sealer tag length (5 measurements/intracanal location/condition) were also recorded from the microtome sections.

Scanning electron microscopy analysis

Matched-half subsections for SEM were treated as follows: 5 N HCl for 30 s; distilled water rinse; 5% NaOCl for 30 min; distilled water rinse; dehydration in ascending grades of ethanol. Following thorough drying (overnight), specimens were mounted on aluminium stubs and sputter-coated with gold–palladium. Specimens were evaluated at 500 and 2000× at 5 kV for secondary electron images and 15 kV for backscatter electron images (Philips XL30 ESEM-FEG, Eindhoven, Netherlands).

Data analyses

Goldner's trichrome stain images were evaluated qualitatively and quantitatively. Linear measurements were analysed using descriptive statistics and a two-factor (condition and intracanal location) repeated measures analysis of variance (ANOVA, $\alpha = 0.05$). Scanning electron images were qualitatively evaluated as a complimentary confirmation for the trichrome staining.

Results

Goldner's trichrome-stained specimens revealed dentine (green) with black/clear stained epoxy resin at the intracanal surface and within the tubules. A conspicuous hybrid layer at the sealer/dentine interface was absent, suggesting a lack of intertubular collagen infiltration in both wet and dry specimens. Consequently, measurements of hybrid layer width and exposed protein were not feasible. Instead, the qualitative examination revealed more uniform intracanal sealer distribution in the coronal and middle thirds with poor distribution in the apical third, in both dry and wet groups (Fig. 2).

Although the sealer did not penetrate the intertubular collagen, sealer penetrated the tubules with depth measurements from GTS images in Table 1. Despite a high degree of tubule penetration variability, there was a significant effect of condition ($P < 0.05$) with deeper penetration in the dry group when compared to the wet. There was also a significant effect of intracanal location ($P < 0.05$) with deeper penetration in the coronal (Fig. 2a,b) and middle thirds (Fig. 2c,d)

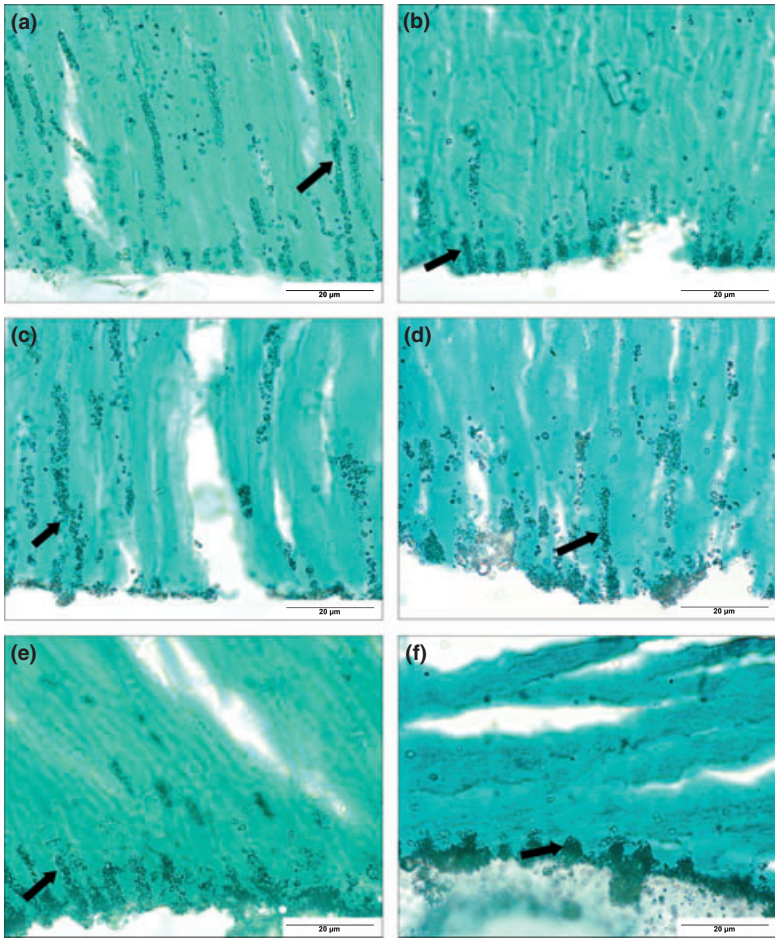

Figure 2 Goldner's trichrome stain images of dry (left) and wet (right) specimens with respective regional sections (a, b) coronal, (c, d) middle, (e, f) apical (100×). Dentine (green) with no evident hybrid layer; resin sealer (clear/black) at canal interface and within dentin tubules (arrows). Greater resin tubule depth in coronal and middle thirds with deeper penetration in dry specimens.

Table 1 Epoxy resin sealer tubule penetration [mean (SD)] as a function of condition and intracanal location

Condition (n = 15)	Coronal (μm)	Middle (μm)	Apical (μm)	Means Across Location*
Dry	31.01 (15.28)	24.15 (24.34)	13.74 (7.59)	22.97
Wet	10.00 (7.00)	13.27 (13.73)	4.88 (4.27)	9.38
Means Across Condition⁺	20.51	18.71	9.31	

*Significant difference because of condition across location ($P < 0.05$) with deeper penetration in dry specimens.
⁺Significant difference because of intracanal location across conditions ($P < 0.05$) with deeper penetration in coronal and middle thirds.
n = 15 (five measurements/intracanal location/condition, three teeth per condition).

compared to the apical third (Fig. 2e,f), irrespective of moisture.

The SEM analysis confirmed GTS findings, demonstrating no visible hybrid layer and a trend towards deeper tubular sealer penetration in the dry versus wet specimens, especially in the coronal and middle thirds (Fig. 3). As with GTS images, the SEM images demonstrated minimal penetration in the relatively atubular apical third of the dry specimen (Fig. 3e). However, there appeared to be no tubule penetration in the wet apical SEM images (Fig. 3f). This is in contrast to the GTS wet apical image (Fig. 2f), which demonstrated minimal tubule penetration.

In addition, the SEM analysis demonstrated the presence of large resin sealer filler particles, some with diameters similar to or greater than the dentinal tubule diameter (Fig. 4). Interestingly, the SEM analysis also revealed sclerotic casts almost totally or

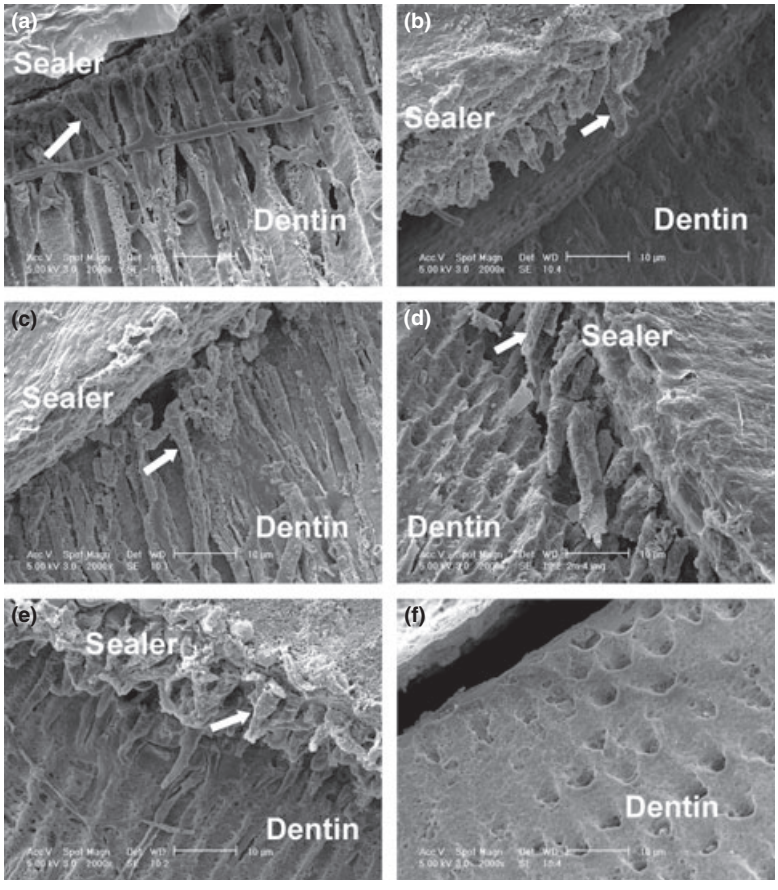

Figure 3 Scanning electron microscopy images of dry (left) and wet (right) specimens with respective regional sections (a, b) coronal, (c, d) middle, (e, f) apical (2000×). A hybrid layer is not evident across condition or location. Within dry specimens, resin tubule penetration tends to be deeper in coronal and middle thirds compared to the apical third. With wet specimens, some resin tubule penetration is evident in coronal and middle thirds, with no apparent tubule penetration in the apical third. Arrows indicate sealer tubular tags.

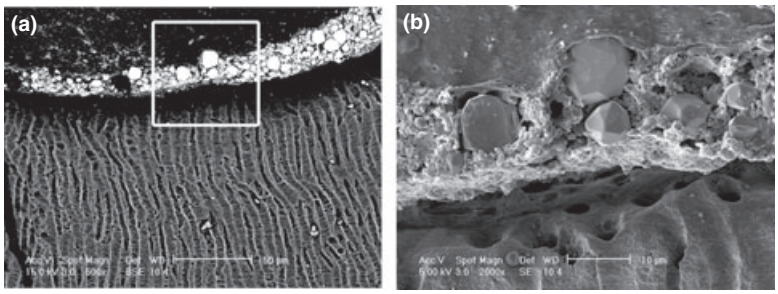

Figure 4 Scanning electron microscopy images demonstrating filler particles blocking dentinal tubule openings. (a) Backscatter image at 500×, (b) 2000× magnification of image an inset.

partially occluding the dentinal tubules in three of six tooth specimens (one wet, two dry). The casts (Fig. 5a) appear similar to tubular resin tags (Fig. 5b) in secondary electron images. However, by using backscatter electron imaging that is sensitive to compositional differences, the casts could be differentiated from resin tags because of the presence of filler particles in the tags (Fig. 5d) but not in the casts (Fig. 5c).

As no hybrid layer could be observed, there was no difference in sealer intertubular collagen infiltration as a function of drying techniques and intracanal location (coronal, middle, apical). In contrast, the results showed a significant difference ($P < 0.05$) in sealer tubule penetration as a function of drying technique and intracanal location, with increased tubule penetration under dry conditions and in the coronal and middle thirds compared to the apical third.

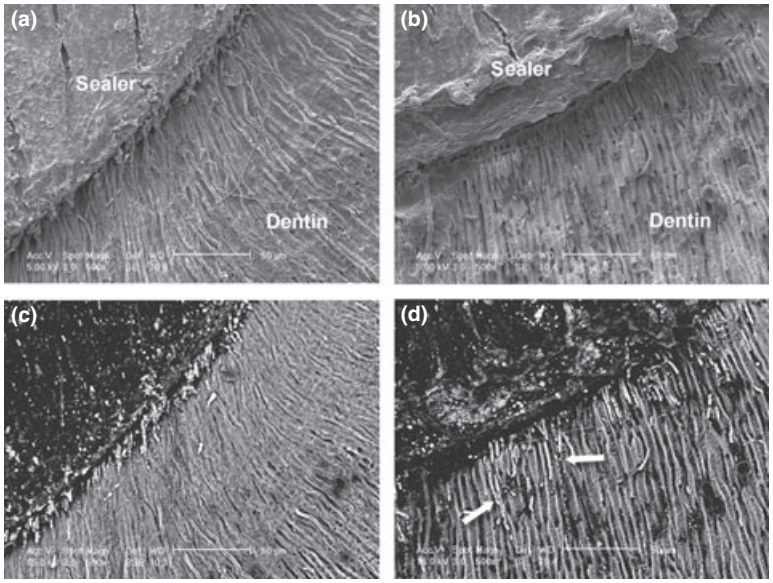

Figure 5 Scanning electron microscopy images demonstrating sclerotic casts or resin tags. (a) Secondary electron image of sclerotic casts (500×); (b) Secondary electron image of tubule resin tags (500×); (c) Backscatter photomicrograph of image a with no radio-opaque particles evident within the tubules; (d) Backscatter photomicrograph of image b demonstrating radio-opaque particles associated with resin within tubules (arrows).

Discussion

In Restorative Dentistry when the demineralized collagen network is over-dried, the quality of adhesive resin/dentine interface can be adversely affected. When dried, the demineralized dentine may collapse up to 65% in volume, effectively impeding desirable resin infiltration of the collagen network (Carvalho *et al.* 1996). Despite this evidence suggesting that some residual moisture may be required with resin-based endodontic sealers, many clinicians desiccate the root canal system prior to sealer application, irrespective of sealer type. Because the effect of canal drying on the quality of the sealer/dentine interface with resin-based sealers is largely unknown, the goal of this pilot study was to apply experimental methods used to evaluate restorative adhesive systems to determine how different drying techniques, in conjunction with intracanal location (coronal, middle, apical), impact on the ability of resin-based sealers to effectively infiltrate both intertubular collagen and dentinal tubules.

In the current study, both GTS and SEM failed to demonstrate a conspicuous hybrid layer at the epoxy resin/dentine interface. This is in agreement with a previous study in which a hybrid layer was not demonstrated with a transmission electron microscopic (TEM) evaluation of the epoxy resin sealer/dentine interface (Tay *et al.* 2005). There are several possible reasons for the lack of hybridization observed under both wet and dry conditions in the current study. After instrumentation, when chelating solutions such as EDTA are used as a final rinse, the radicular dentine is affected, leaving a microporous demineralized collagen matrix (Tay *et al.* 2007) requiring the presence of residual water molecules to prevent the formation of interfibrillar hydrogen bonds, leading to collagen network collapse (Pashley *et al.* 2007). However, the resin sealer used in this study is highly filled with macro/microfillers, which may limit the resin infiltration of the collagen matrix even if it was fully expanded under the wet conditions of this study. It has been demonstrated that the collagen matrix is not penetrable by fillers, even nanofillers in dentine adhesives (Tay *et al.* 1999).

Further, it is also possible that hybrid layer formation may be inhibited when the dentinal collagen has been denatured. It has been reported that either hydrated or partially dehydrated dentinal collagen partially demineralized by mild acid or chelating agents may be susceptible to thermal denaturation (Armstrong *et al.* 2006). With temperatures between 200 and 250 °C used with warm vertical compaction in the current study, thermal denaturation is a possibility. However, a previous TEM analysis reported no thermal denaturation of collagen fibrils following warm vertical compaction (Tay *et al.* 2005).

Although the lack of hybridization in this study precluded quantitative measure of the hybrid layer depth, a qualitative examination demonstrated that sealer distribution along the canal wall appeared more uniform in coronal and middle thirds and poor in the apical third in both wet and dry groups. Another study evaluating several different sealer placement tech-

niques reported similar findings (Wiemann & Wilcox 1991); regardless of sealer placement technique, sealer coverage in the coronal and middle thirds was similar, whilst the greatest variability of sealer coverage was in the apical third.

Likewise, sealer tubule penetration depth in this study varied in relation to intracanal location with greater penetration in the coronal and middle thirds. Similar results were reported with epoxy- and methacrylate-based resin sealers with more prominent resin tubule penetration in the coronal and middle thirds and minimal penetration in the apical third. (Sevimay & Kalayci 2005) These intracanal differences can potentially be attributed to the decreasing gradient of tubule density and diameter from coronal to apical aspects of the root. (Carrigan *et al.* 1984, Camargo *et al.* 2007). Nonetheless, these differences in sealer penetration might contribute to a compromised seal and root canal treatment failure, as the sealer matrix physical integrity has been reported to be an important factor to reduce microleakage (Kokkas *et al.* 2004).

In addition to the effect of intracanal location, the results of the current study also demonstrated that residual moisture reduced the epoxy resin sealer tubule penetration depth. Whilst the variability in sealer penetration depth was high, on average the sealer penetrated deeper in dry specimens versus wet specimens and deeper in coronal and middle thirds of the root versus the apical third. Although no previous studies characterized the quality of the sealer/dentine interface as a function of residual moisture, some recent studies evaluated the effect of moisture on dye leakage (Roggendorf *et al.* 2007, Zmener *et al.* 2008). Even though the results of those studies cannot be directly compared to the current study, it was reported that some moisture resulted in the decreased leakage for two methacrylate-based resin sealers (Zmener *et al.* 2008), whilst residual moisture was associated with increased leakage with an epoxy resin sealer, the same product used in the current study (Roggendorf *et al.* 2007). This difference could be associated with the fact that methacrylate-based sealers are more hydrophilic than epoxy-based sealers. If residual moisture translates into decreased sealer penetration with epoxy resin sealer, then this would add additional evidence to corroborate the findings of previous authors (Sen *et al.* 1996, Roggendorf *et al.* 2007) that moisture leads to increased leakage most likely due to less than optimal intracanal distribution and decreased tubule penetration of the sealer.

Another possible reason for the observed variability of sealer tubule depth penetration may be related to the preparation and measurement of the GTS-prepared slides. Sectioning the dentine discs on the microtome might fracture the delicate sealer tags, particularly as they spread into the peripheral dentine. It was also observed that in some specimens (Fig. 1a), the tubules appeared to be discontinuous; however, only continuous portions of the sealer tags were measured. The discontinuity might be explained by the tubule being sectioned as it curved through multiple planes, as the course of tubules follow a gentle S-shaped curve, not a straight line, from the pulp to the tooth periphery (Avery & Chiego 2006).

The SEM analysis revealed another possible reason not only for the poor resin infiltration of the collagen matrix, but for decreased tubular penetration as well. Zirconium oxide and calcium tungstate fillers are added to the sealer to improve viscosity during sealer application and to increase radiopacity (Dentsply-DeTrey-GmbH 2005). The manufacturer reports the average calcium tungstate filler particle diameter at 8 μm. These large diameter particles would likely preclude infiltration of the sealer into the demineralized dentine matrix and the dentinal tubules (Fig. 3), as the average tubule diameters range from 2.5–4 μm (Garberoglio & Brannstrom 1976, Sen *et al.* 1996).

The SEM analysis demonstrated an additional potential impediment to sealer tubular penetration, the relatively high incidence of sclerotic casts in the dentinal tubules. These casts were present in some degree in half the study teeth. Whilst the presence of tubular sclerotic casts especially in coronal dentine has been reported (Nalbandian *et al.* 1960, Weber 1974, Yoshiyama *et al.* 1990, Mixson *et al.* 1995, Tay & Pashley 2004), a review of bonding to coronal sclerotic dentine reported that bond strengths to such dentine were significantly reduced, as was resin tubule infiltration (Tay & Pashley 2004). Although the presence of sclerotic casts in radicular dentine has also been recognized (Nalbandian *et al.* 1960, Eda *et al.* 1996), the implications on endodontic sealer tubule penetration or leakage have not been addressed to date. The current results indicate that the prevalence of such casts significantly reduced sealer tubule penetration depth, which might lead to increased microleakage (Sen *et al.* 1996).

Clinicians currently employ various methods of drying the canal prior to sealing and obturation. Whilst some may simply use numerous paper points, others may attempt to completely desiccate the canal using any combination of a final ethanol rinse, suction, paper points and air-drying. It must be noted, however,

that predictably controlling the level of moisture in root canal systems may not be possible, given the presence of complex canal anatomy, fins, isthmuses and lateral canals. Further, as evidenced in this study and others, residual moisture in the root canal may or may not be beneficial to sealer canal wall coverage, tubular penetration and/or the sealing ability, depending on sealer composition. For example, ZOE, CaOH and methacrylate-containing endodontic sealers seem to benefit from some moisture (Kuhre & Kessler 1993, Horning & Kessler 1995, Roggendorf *et al.* 2007, Zmener *et al.* 2008), whilst epoxy-based resin sealers, as observed in the present study, perform better with thorough canal drying. Thus, clinicians should know the properties of the materials they use and adjust their treatment protocols accordingly.

In the current study, the complimentary GTS and SEM techniques proved to be beneficial experimental methods for evaluating the root canal system. For instance, SEM demonstrated the presence of large filler particles and sclerotic casts that were not apparent using GTS evaluation, whilst GTS demonstrated sealer tags in apical sections where they were not apparent in the matched SEM section, likely due to being lost during specimen preparation. However, because this was an initial, exploratory pilot study, the greatest investigation limitation was the relatively small number of evaluated specimens, which may limit direct clinical applicability and might preclude definitive conclusions regarding moisture. A future study with a larger sample size is necessary and with a larger sample, additional levels of moisture could be evaluated, from saturation, to intermediate moisture, to desiccated dentine along with the evaluation of additional types of resin sealer, such as methacrylate-based resins. Moreover, analytical techniques such as micro-Raman spectroscopy, a non-destructive methodology, could be used to provide a sealer/dentine interface chemical characterization that can include information such as the degree of resin polymerization and can be carried out on the same specimens prior to the morphologic evaluation with SEM or GTS.

Conclusions

The results of this pilot study suggest that the experimental methodology (combination of GTS and SEM) used to evaluate the coronal resin/dentine interface associated with Restorative Dentistry can also be used to evaluate the intracanal resin sealer/dentine interface. Based on the pilot data, there was no observable intertubular collagen infiltration of the epoxy resin sealer and consequently, no difference in the degree of intertubular collagen infiltration, irrespective of drying technique or intracanal location (coronal, middle and apical). Moisture appeared to significantly reduce the degree of tubule penetration of the epoxy resin sealer. Tubule penetration of the sealer was also significantly deeper in coronal and middle thirds and minimal in the apical third, irrespective of drying technique.

Acknowledgements

The authors thank Dr Vladimir Dusevich for his assistance with the SEM analysis and Dentsply/Tulsa Dental for contributing the AH Plus sealer for this study.

References

Armstrong SR, Jessop JL, Winn E, Tay FR, Pashley DH (2006) Denaturation temperatures of dentin matrices. I. Effect of demineralization and dehydration. *Journal of Endodontics* **32**, 638–41.

Avery JK, Chiego DJ (2006) Dentin. In: Piesco NP, ed. *Essentials of Oral Histology and Embryology*, 3rd edn. St Louis, MO: Elsevier Mosby, pp. 108–9.

Calt S, Serper A (2002) Time-dependent effects of EDTA on dentin structures. *Journal of Endodontics* **28**, 17–9.

Camargo CH, Siviero M, Camargo SE, de Oliveira SH, Carvalho CA, Valera MC (2007) Topographical, diametral, and quantitative analysis of dentin tubules in the root canals of human and bovine teeth. *Journal of Endodontics* **33**, 422–6.

Carrigan PJ, Morse DR, Furst ML, Sinai IH (1984) A scanning electron microscopic evaluation of human dentinal tubules according to age and location. *Journal of Endodontics* **10**, 359–63.

Carvalho RM, Yoshiyama M, Pashley EL, Pashley DH (1996) In vitro study on the dimensional changes of human dentine after demineralization. *Archives of Oral Biology* **41**, 369–77.

Dentsply-DeTrey-GmbH (2005) *AH Plus Root Canal Sealer: Scientific Compendium*. Konstanz, Germany.

Doyle MD, Loushine RJ, Agee KA *et al.* (2006) Improving the performance of EndoRez root canal sealer with a dual-cured two-step self-etch adhesive. I. Adhesive strength to dentin. *Journal of Endodontics* **32**, 766–70.

Eda S, Hasegawa H, Kawakami T (1996) Crystals closing tubules in sclerosed root dentin of the aged. In: *Proceedings of the International Conference on Dentin/Pulp Complex 1995 and the International Meeting on Clinical Topics of Dentin/Pulp Complex*. Osaka, Japan: Quintessence Publishing.

Garberoglio R, Brannstrom M (1976) Scanning electron microscopic investigation of human dentinal tubules. *Archives of Oral Biology* **21**, 355–62.

Gharib SR, Tordik PA, Imamura GM, Baginski TA, Goodell GG (2007) A confocal laser scanning microscope investigation of the epiphany obturation system. *Journal of Endodontics* **33**, 957–61.

Goldman M, Goldman LB, Cavaleri R, Bogis J, Lin PS (1982) The efficacy of several endodontic irrigating solutions: a scanning electron microscopic study: Part 2. *Journal of Endodontics* **8**, 487–92.

Horning TG, Kessler JR (1995) A comparison of three different root canal sealers when used to obturate a moisture-contaminated root canal system. *Journal of Endodontics* **21**, 354–7.

Kokkas AB, Boutsioukis A, Vassiliadis LP, Stavrianos CK (2004) The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers: an in vitro study. *Journal of Endodontics* **30**, 100–2.

Kuhre AN, Kessler JR (1993) Effect of moisture on the apical seal of laterally condensed gutta-percha. *Journal of Endodontics* **19**, 277–80.

Mixson JM, Spencer P, Moore DL, Chappell RP, Adams S (1995) Surface morphology and chemical characterization of abrasion/erosion lesions. *American Journal of Dentistry* **8**, 5–9.

Nalbandian J, Gonzales F, Sognnaes RF (1960) Sclerotic age changes in root dentin of human teeth as observed by optical, electron, and x-ray microscopy. *Journal of Dental Research* **39**, 598–607.

Pashley DH, Tay FR, Carvalho RM *et al.* (2007) From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. *American Journal of Dentistry* **20**, 7–20.

Plasmans PJ, Creugers NH, Hermsen RJ, Vrijhoef MM (1994) Intraoral humidity during operative procedures. *Journal of Dentistry* **22**, 89–91.

Roggendorf MJ, Ebert J, Petschelt A, Frankenberger R (2007) Influence of moisture on the apical seal of root canal fillings with five different types of sealer. *Journal of Endodontics* **33**, 31–3.

Schwartz RS (2006) Adhesive dentistry and endodontics. Part 2: bonding in the root canal system-the promise and the problems: a review. *Journal of Endodontics* **32**, 1125–34.

Sen BH, Piskin B, Baran N (1996) The effect of tubular penetration of root canal sealers on dye microleakage. *International Endodontic Journal* **29**, 23–8.

Sevimay S, Kalayci A (2005) Evaluation of apical sealing ability and adaptation to dentine of two resin-based sealers. *Journal of Oral Rehabilitation* **32**, 105–10.

Spencer P, Swafford JR (1999) Unprotected protein at the dentin-adhesive interface. *Quintessence International* **30**, 501–7.

Spencer P, Wang Y, Walker MP, Wieliczka DM, Swafford JR (2000) Interfacial chemistry of the dentin/adhesive bond. *Journal of Dental Research* **79**, 1458–63.

Tay FR, Pashley DH (2004) Resin bonding to cervical sclerotic dentin: a review. *Journal of Dentistry* **32**, 173–96.

Tay FR, Moulding KM, Pashley DH (1999) Distribution of nanofillers from a simplified-step adhesive in acid-conditioned dentin. *Journal of Adhesive Dentistry* **1**, 103–17.

Tay FR, Loushine RJ, Weller RN *et al.* (2005) Ultrastructural evaluation of the apical seal in roots filled with a polycaprolactone-based root canal filling material. *Journal of Endodontics* **31**, 514–9.

Tay FR, Gutmann JL, Pashley DH (2007) Microporous, demineralized collagen matrices in intact radicular dentin created by commonly used calcium-depleting endodontic irrigants. *Journal of Endodontics* **33**, 1086–90.

Van Meerbeek B, De Munck J, Yoshida Y *et al.* (2003) Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. *Operative Dentistry* **28**, 215–35.

Walker MP, Wang Y, Spencer P (2002) Morphological and chemical characterization of the dentin/resin cement interface produced with a self-etching primer. *Journal of Adhesive Dentistry* **4**, 181–9.

Wang Y, Spencer P (2004) Physiochemical interactions at the interfaces between self-etch adhesive systems and dentine. *Journal of Dentistry* **32**, 567–79.

Wang Y, Spencer P, Hager C, Bohaty B (2006) Comparison of interfacial characteristics of adhesive bonding to superficial versus deep dentine using SEM and staining techniques. *Journal of Dentistry* **34**, 26–34.

Weber DF (1974) Human dentine sclerosis: a microradiographic survey. *Archives of Oral Biology* **19**, 163–9.

White RR, Goldman M, Lin PS (1984) The influence of the smeared layer upon dentinal tubule penetration by plastic filling materials. *Journal of Endodontics* **10**, 558–62.

Wiemann AH, Wilcox LR (1991) In vitro evaluation of four methods of sealer placement. *Journal of Endodontics* **17**, 444–7.

Yamada RS, Armas A, Goldman M, Lin PS (1983) A scanning electron microscopic comparison of a high volume final flush with several irrigating solutions: Part 3. *Journal of Endodontics* **9**, 137–42.

Yoshiyama M, Noiri Y, Ozaki K, Uchida A, Ishikawa Y, Ishida H (1990) Transmission electron microscopic characterization of hypersensitive human radicular dentin. *Journal of Dental Research* **69**, 1293–7.

Zmener O, Spielberg C, Lamberghini F, Rucci M (1997) Sealing properties of a new epoxy resin-based root-canal sealer. *International Endodontic Journal* **30**, 332–4.

Zmener O, Pameijer CH, Serrano SA, Vidueira M, Macchi RL (2008) Significance of moist root canal dentin with the use of methacrylate-based endodontic sealers: an in vitro coronal dye leakage study. *Journal of Endodontics* **34**, 76–9.

