Australian Endodontic Journal
The Journal of the Australian Society of Endodontology Inc.,
the Australian and New Zealand Academy of Endodontists and
the Asian Pacific Endodontic Confederation

Aust Endod J 2017

ORIGINAL RESEARCH

Sealer penetration and adaptation in root canals with the butterfly effect

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Keywords
dentine, endodontics, root canal sealers.

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doi: 10.1111/aej.12238

(Accepted for publication 9 September 2017.)

Abstract

The butterfly effect is a phenomenon seen in some roots and is related to density of dentinal tubules. The aim was to investigate penetration depth and adaptation quality of root canal sealers and ProRoot MTA into bucco-lingual and mesio-distal aspects of roots with and without the effect. One hundred and twenty teeth were decoronated at the cemento-enamel junction. Canals were prepared and assigned to obturation groups: gutta-percha with a sealer (AH Plus, EndoREZ, Kerr Pulp Canal Sealer, MTA Fillapex) or ProRoot MTA alone (each containing 10 butterfly and 10 non-butterfly roots). Root sectioning yielded coronal and middle samples. Confocal laser scanning and scanning electron microscopy were used to assess penetration and adaptation. Teeth with the effect had greater mean penetration bucco-lingually (766 μm) than mesio-distally (184 μm, $P = 0.003$). Coronal sections had greater penetration (430 μm) compared with middle (247 μm, $P = 0.006$). In conclusion, greater penetration in roots with the effect may improve treatment outcomes.

Introduction

Chronic apical periodontitis is a sequel to bacterial infection of the pulp and root canal system (1). The principle goal of root canal treatment is the elimination of disease-causing microbes from the root canal system. The use of a sealer during obturation is important to minimise voids between the core filling material and the canal wall, and to seal dentinal tubules and lateral canals. In the absence of a sealer, root canal fillings may leak, leading to failure of treatment. Penetration refers to the amount of sealer entering the dentinal tubules and adaptation quality describes the way in which the sealer conforms to the dentine wall. These are important properties of the many products on the market.

Penetration and adaptation depends on many factors including the patency and density of the dentinal tubules. Some teeth exhibit an optical phenomenon known as the “Butterfly Effect” (2) and have a significantly higher density of dentinal tubules in the bucco-lingual direction compared with the mesio-distal (3). This produces a characteristic butterfly shape (Fig. 1) in transverse sections of the roots caused by the different shades of dentine (2–4). Sclerotic dentine causes light to refract and scatter (5). A decrease in the number of dentinal tubules results in greater light transmission to give a translucent appearance (6).

The butterfly effect may impact on the behaviour of sealers inside root canals (3). A PubMed review of the literature from 1931 to 2017 reveals no studies on the topic of sealer penetration and adaptation in teeth with the butterfly effect. The aim of this study was to investigate the penetration and adaptation of common types of root canal sealers (AH Plus, Kerr Pulp Canal Sealer, MTA Fillapex and EndoREZ) and the obturation material (ProRoot MTA) in cross-sections of tooth roots exhibiting the butterfly effect and to determine if this differs between coronal and middle root sections. It was hypothesised that teeth with the butterfly effect will have greater sealer penetration bucco-lingually and that coronal sections will have greater penetration than middle sections.

Materials and methods

Power calculations were carried out to determine an appropriate sample size. Ethical approval was granted from the University of Otago, Dunedin, New Zealand (reference H15/077) to collect 120 permanent, maxillary

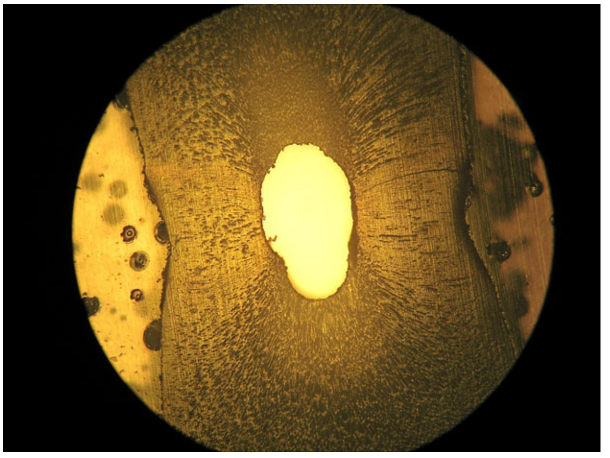

Figure 1 Root section (×10) under light microscope showing the butterfly shape.

anterior human teeth. Teeth of known patient age (25–40 years) were used. Teeth with root resorption, immature apices, fracture or root filling were rejected. Teeth were washed and stored in phosphate buffered saline (PBS, pH: 7.4) until required. They were decoronated at the cemento-enamel junction with a diamond bur and viewed under a light microscope (EHT; Olympus, Tokyo, Japan) at ×10 magnification and coded 'B' butterfly or 'NB' non-butterfly according to the presence or absence of the effect (Fig. 1).

All preparations were completed using a dental operating microscope (DOM; OPMI pico, Carl Zeiss Ltd, Oberkochen, Germany) at ×6 magnification. The working length of the roots was determined visually by subtracting 1 mm from the point at which a size 10 K-file was seen at the major apical foramen. Canal orifices were flared with X-Gates files (Dentsply Tulsa Dental, Tulsa, OK, USA) and prepared using ProTaper Next (Dentsply Maillefer, Ballaigues, Switzerland) instruments to size X3. Copious irrigation and recapitulation with 5.25% sodium hypochlorite was carried out, and apical patency was maintained with a size 10 K-file. Prior to obturation, the canals were irrigated with 5 mL of EDTA (EDTA 15%; Ultradent, South Jordan, UT, USA) solution for 2 min then 5 mL of 5.25% NaOCl for another 2 min to remove organic material and cutting debris. Canals were finally rinsed with 0.9% saline and dried with paper points. Roots were randomly assigned to five obturation groups; gutta-percha (GP) with AH Plus sealer (Dentsply DeTrey, Konstanz, Germany), GP with EndoREZ sealer (Ultradent), GP with Pulp Canal Sealer (Kerr, Romulus, MI, USA), GP with MTA Fillapex sealer (Angelus, Parana, Brazil), and white ProRoot MTA alone (Dentsply Tulsa Dental). Each group comprised twenty roots (10 B and 10 NB). A control group of ten B and ten NB prepared but unfilled roots was used to confirm smear layer removal.

To provide fluorescence for confocal laser scanning microscopy (CLSM), the sealers and ProRoot MTA were mixed with rhodamine B dye (Sigma-Aldrich, St Louis, MO, USA) at a 1:100 ratio by weight. Sealer was placed in the canals using a size 15 K file at the working length with a counter-clockwise motion. Obturation was with single ProTaper Next X3 GP cones with excess GP removed with a heated instrument and vertically condensed. MTA was placed using the MAP system (Dentsply Tulsa Dental) and condensed using Buchanan pluggers (Kerr). Filled roots were stored in 100% humidity at 37°C for 2 weeks to allow complete set of materials.

Roots were embedded in acrylic resin (Vertex™ Castapress, Vertex-Dental, Zeist, The Netherlands) in plastic cuvettes (LP Italiana SpA, Milan, Italy). An Accutom 50 precision slicing machine (Struers A/S, Ballerup, Denmark) fitted with an Accutom 50 blade MOD13 (Struers) was used to section roots at distances 8 and 11 mm from the apex to yield coronal and middle sections of root. To remove surface defects, sections were polished with a TegraPol 21 polishing machine (Struers) using silicon carbide paper (P1200–P4000; 3M Europe, Diegem, Belgium) and sterile water. To remove debris, specimens were rinsed with 15% EDTA and 5.25% NaOCl for 2 min.

Root sections were first viewed using CLSM (Zeiss LSM 510, Axioplan 200, Carl Zeiss Ltd., Jena, Germany) and bird's eye-view images (×10) were taken. A total of 200 images were captured corresponding to 100 coronal and 100 middle root sections. A superimposed grid was used to standardise direction records (Fig. 2). The depth of penetration (μm) was measured at 12 points using the measuring tool in Image J (National Institute of Health, Bethesda, MD, USA). Mean sealer penetration scores were calculated for each direction (bucco-lingual versus mesio-distal) at two root levels (coronal and middle).

Root sections were then observed using scanning electron microscopy (SEM; JEOL JSM 6700F, JEOL Ltd, Tokyo, Japan). Micrographs (×400) were taken of each coronal and middle root section at the dentine sealer or ProRoot MTA interface. The quality of sealer or ProRoot MTA adaptation to the intracanal dentine at four points (buccal, lingual, mesial and distal) was scored as good, reasonable, poor or absent (Table 1).

All CLSM and SEM images were assessed by an examiner (AR). Fifteen of the CLSM and SEM images were further assessed by two calibrated and independent specialist endodontists. Observers viewed the images on a computer after receiving written instructions and familiarisation. All three assessors were unaware which root section type was under consideration.

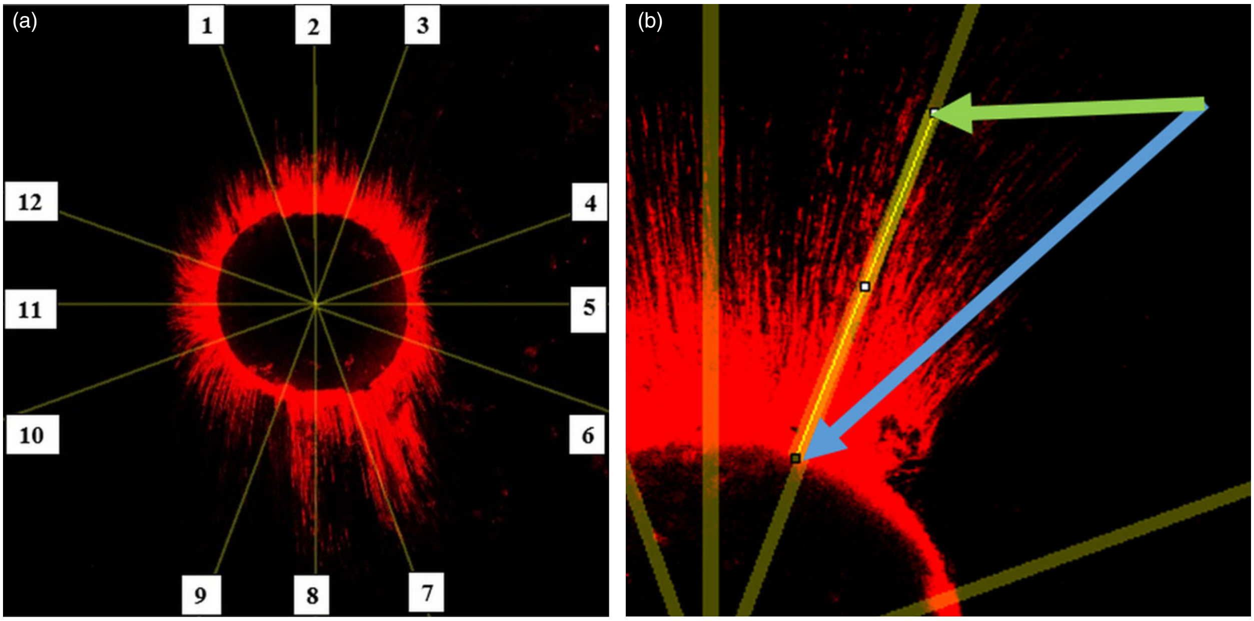

Figure 2 (a) Confocal image (×10) of a root section showing assessment grid. Numbers correspond to direction as follows 1–3 (buccal), 4–6 (mesial), 7–9 (lingual) 10–12 (distal). (b) Example of measuring tool used to draw a line from the dentine-sealer interface (blue arrow) to outermost red point along the gridline (green arrow) representing sealer penetration.

Table 1 Sealer adaptation descriptors. Adapted from (11)

Adaptation quality	Description
Good	The section showed no gaps between sealer and dentine. Sealer can be seen penetrating into dentinal tubules
Reasonable	The section shows some small gaps (<10 μm) between sealer and dentine
Poor	The section shows many gaps (>10 μm) between the sealer and dentine
Absent	The majority of the section shows no adaptation between the sealer and dentine

Statistical analyses were performed using Stata version 13.1 (StataCorp. College Station, TX, USA). The data were analysed using a mixed model (ANOVA). The significance level was set at $P < 0.05$. A Kappa test was used to determine inter-examiner reliability.

Results

Penetration of sealers and ProRoot MTA

Representative confocal images (×10) of penetration are shown in Figures 3 and 4 and results are presented in Tables 2 and 3. Teeth with the butterfly effect had greater penetration bucco-lingually (766 μm) compared with mesio-distally (184 μm), a significant difference ($P = 0.003$). In contrast, teeth without the butterfly effect had no significant difference between bucco-lingual (385 μm) and mesio-distal (387 μm) penetrations ($P = 0.98$).

Teeth with the butterfly effect had significantly greater penetration bucco-lingually (766 μm) compared with teeth without the effect (385 μm; $P = 0.01$). On the other hand, teeth with the butterfly effect had significantly less penetration mesio-distally (184 μm) compared with teeth without the effect (387 μm; $P = 0.008$).

Coronal sections had the best mean penetration (430 μm) compared with middle sections (247 μm), a significant difference ($P = 0.006$). Mean penetration in middle sections was significantly less in teeth with the butterfly effect (162 μm) compared with teeth without the effect (332 μm; $P = 0.04$).

Penetration varied between obturation material groups but this did not reach significance ($P = 0.23$). In order of decreasing penetration; AH Plus > MTA Fillapex > EndoREZ > Pulp Canal Sealer > ProRoot MTA. Agreement between the three examiners was substantial (Kappa = 0.61).

Adaptation of sealers and ProRoot MTA

Representative SEM micrographs (×400) of sealer adaptation are shown in Figure 5. Adaptation of ProRoot MTA and crystal growth observed in dentinal tubules are

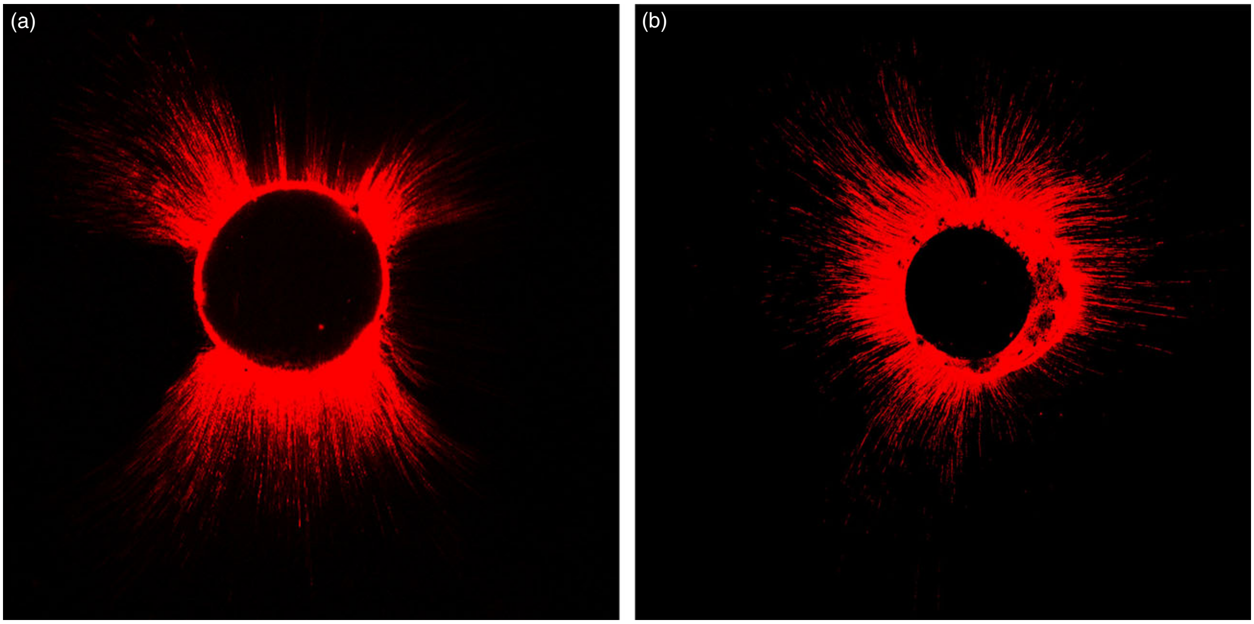

Figure 3 Side-by-side comparison of representative confocal laser scanning microscopy images (×10) of penetration of AH Plus sealer in roots with the butterfly effect (a) and without the effect (b).

shown in Figure 6. The SEM results appear in Table 4. Adaptation was more favourable in coronal sections (78% good or reasonable) than middle sections (57% good or reasonable), a significant difference ($P = 0.0012$). In coronal sections, teeth with the butterfly effect had a similar percentage of poor or absent adaptation (10%) compared to teeth without the effect (12%). Middle sections also showed this trend (22% and 23%, respectively). For both butterfly and non-butterfly teeth in coronal and middle sections, there was no significant difference in the quality of adaptation in the bucco-lingual and mesio-distal direction ($P = 0.56$). Similarly, there was no significant difference between butterfly and non-butterfly teeth ($P = 0.20$).

Adaptation quality varied with obturation groups; AH Plus > ProRoot MTA > MTA Fillapex > Pulp Canal Sealer > EndoREZ, however, this did not reach significance ($P = 0.40$). Agreement between the three examiners was substantial (Kappa = 0.68).

Discussion

This study shows that the presence of the butterfly effect influences the penetration of sealers and ProRoot MTA into dentinal tubules. Teeth with the effect consistently showed significantly deeper penetration in a bucco-lingual direction compared with teeth without the effect. This trend was evident despite no significant difference in bucco-lingual and mesial-distal adaptation of sealers and ProRoot MTA to the canal wall. Bacteria are able to remain viable within dentinal tubules creating a reservoir of residual infection (7). Deep penetration of sealer enhances entombment of any remaining microbes and creates an unfavourable environment for microbial growth (8). Furthermore, the deeper a sealer can penetrate into dentinal tubules the greater its antibacterial potential may be (9). Therefore, the inferior penetration of sealers and ProRoot MTA mesio-distally may negatively impact the outcome of root canal treatment in teeth with the butterfly effect. Similarly, it is possible that the penetration of intracanal medicaments such as calcium hydroxide and luting agents used during post cementation could be influenced by the presence of the butterfly effect. Further studies are necessary to confirm this.

Sealer penetration studies should consider the butterfly effect as a potential confounder. Ideally they should specify whether the teeth included have the effect, and if so measurements should be limited to the bucco-lingual direction. This may explain the reported wide range of penetration depths (23–2000 μm) of sealers in some previous studies (10–12).

At present there are no studies on the prevalence of the butterfly effect. One study examined the different characteristics of root sections and described the presence of the butterfly effect as ‘frosted dentin’ which was reported to be more common in premolars and molars than in anterior teeth (13). The clinical significance of

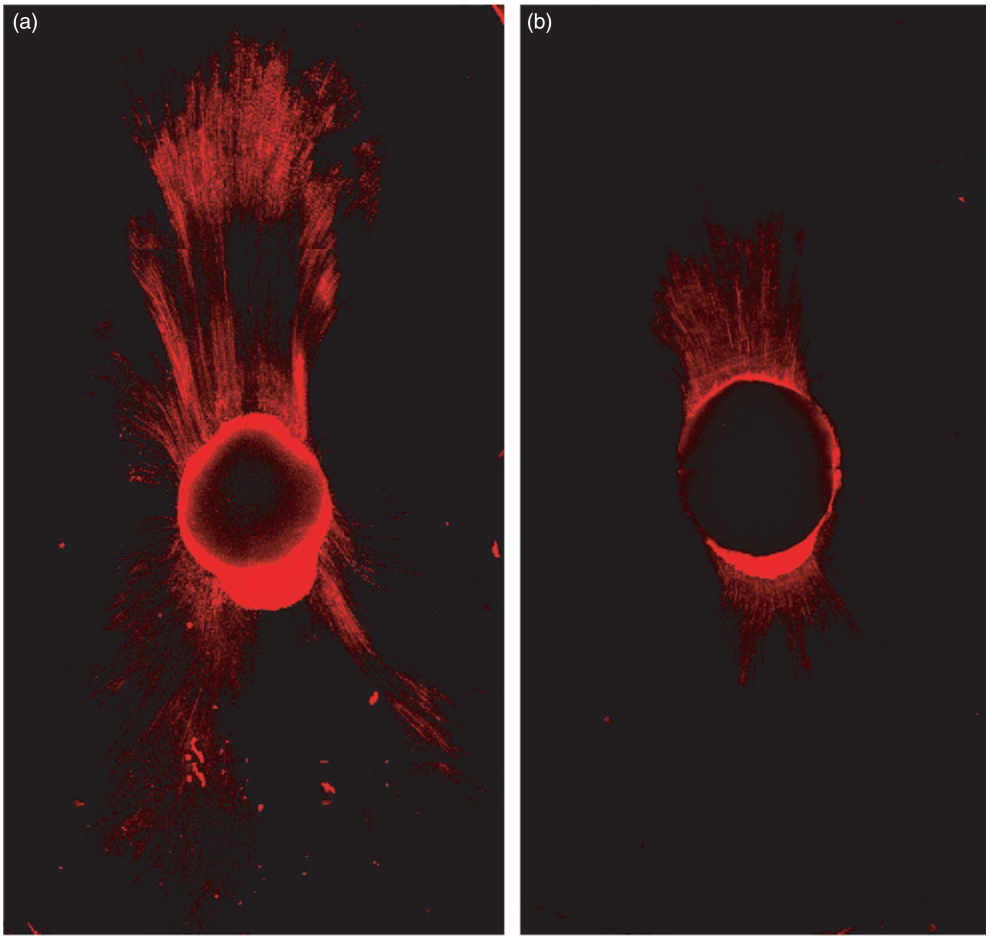

Figure 4 Representative confocal laser scanning microscopy images (×10) showing side-by-side comparison of coronal (a) and middle (b) penetration of AH Plus sealer in a root with the butterfly effect.

Table 2 Mean penetration (μm) in coronal sections of roots with and without the butterfly effect (CLSM)

			Penetration (μm)		
	Obturation group	Sample (*n*)	Bucco-lingual	Mesio-distal	Overall mean
Butterfly	GP with EndoRez	10	747	157	452
	GP with AH Plus	10	1013	185	599
	GP with MTA Fillapex	10	970	320	645
	GP with Pulp Canal Sealer	10	629	106	367
	ProRoot MTA	10	470	151	311
Non-butterfly	GP with EndoRez	10	365	352	358
	GP with AH Plus	10	549	552	551
	GP with MTA Fillapex	10	405	412	409
	GP with Pulp Canal Sealer	10	314	316	315
	ProRoot MTA	10	293	301	297

Table 3 Mean penetration (μm) in middle sections of roots with and without the butterfly effect (CLSM)

			Penetration (μm)		
	Obturation group	Sample (*n*)	Bucco-lingual	Mesio-distal	Overall mean
Butterfly	GP with EndoRez	10	538	39	288
	GP with AH Plus	10	962	61	511
	GP with MTA Fillapex	10	754	86	420
	GP with Pulp Canal Sealer	10	507	60	284
	ProRoot MTA	10	254	58	156
Non-butterfly	GP with EndoRez	10	153	162	158
	GP with AH Plus	10	304	298	301
	GP with MTA Fillapex	10	155	158	157
	GP with Pulp Canal Sealer	10	102	98	100
	ProRoot MTA	10	91	94	93

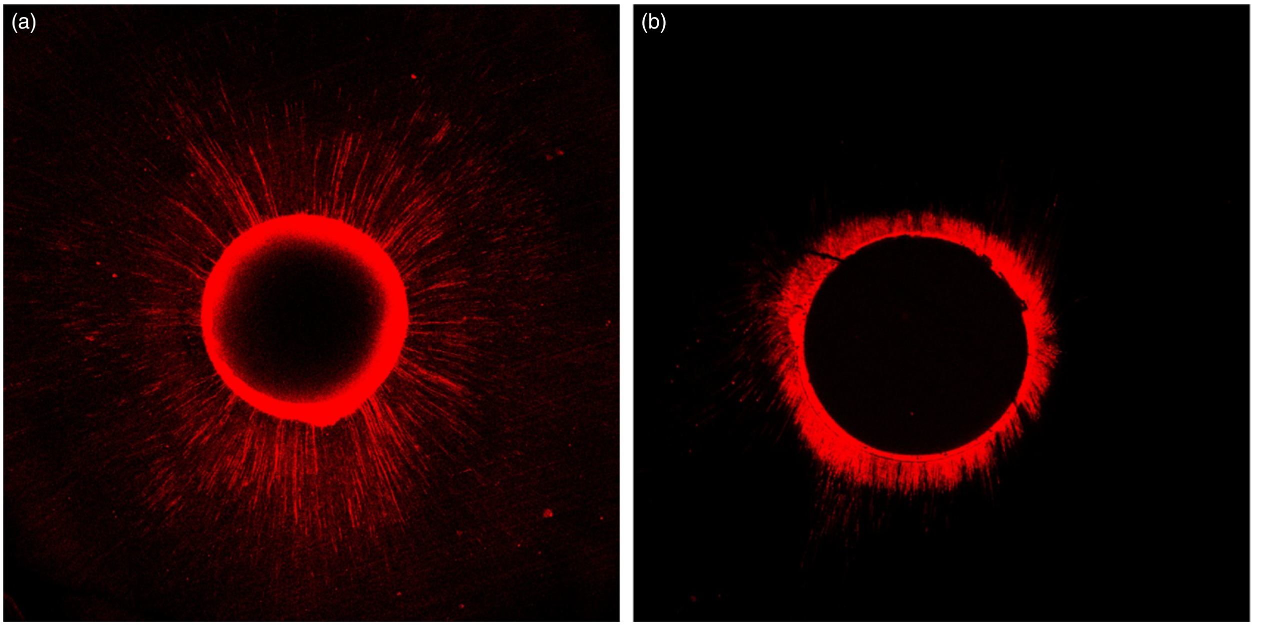

Figure 5 Representative confocal laser scanning microscopy images (×10) showing side-by-side comparison of coronal (a) and middle (b) penetration of AH Plus sealer in a root without the butterfly effect.

the butterfly effect may be greater in posterior teeth. In the present study, only maxillary anterior teeth of similar age were included. The number of dentinal tubules is reported to decrease significantly with increasing age (14) and this may influence the penetration of sealers.

A potential limitation of this study is that teeth were obturated immediately after canal preparation, which is the protocol for single visit treatment but not always a true representation of the clinical situation. Calcium hydroxide dressings in the root canal can affect the depth of sealer penetration as it is not always possible to completely remove this material before obturation (15). The penetration depths reported in this *in vitro* study may be higher than what can be expected clinically when multiple visit treatment is performed.

This study found that adaptation quality and penetration varied between sealers, but no significant difference was found. AH Plus had superior adaptation and penetration. This finding is in agreement with results of previous studies (11,16) and may be attributed to its pseudoplastic behaviour inside root canals. This has been described as a decrease in viscosity and an increase in flow parallel to an increase in shear rate during filling procedures (17). Fillapex MTA also displays this.

The adaptation of ProRoot MTA may be explained by its setting reaction which causes expansion and could enhance the seal with the canal wall. However, despite having the second best adaptation, ProRoot MTA had the least depth of penetration. Several factors may have contributed to this, such as MTA's particle size and intratubular mineralisation. An SEM study compared the tubular penetration of ProRoot MTA with a new obturation material (Capasio) which has half the particle size (18). Penetration of Capasio into dentinal tubules was reported while ProRoot MTA was not (18). The particle size of ProRoot MTA may limit its ability to penetrate deep into dentinal tubules. The average particle size of white ProRoot MTA is 10 μm, with all particles smaller than 50 μm (19) whereas the average particle size of Capasio is 5.3 μm, with all particles smaller than 20 μm (18). However, it is important to note that small particle size is not necessarily an indicator of better penetration. For example, AH Plus, which has a mixed particle size of 26 μm had the best penetration.

MTA is said to form mineralised crystals which grow within the dentinal tubules over time (20). The formation of crystal-like structures inside dentinal tubules was observed in high magnification SEM micrographs in this study (Fig. 7). This mineralisation effect may explain the initial low penetration of MTA into dentinal tubules. In this study, penetration was visualised 2 weeks after obturation. Continued crystal growth has been reported for up to 16 weeks after obturation (20). It is possible that an enhanced effect may be observed with further crystal growth over time. More research is required to investigate this theory.

The variation in the penetration results between different sealers and ProRoot MTA may be influenced by

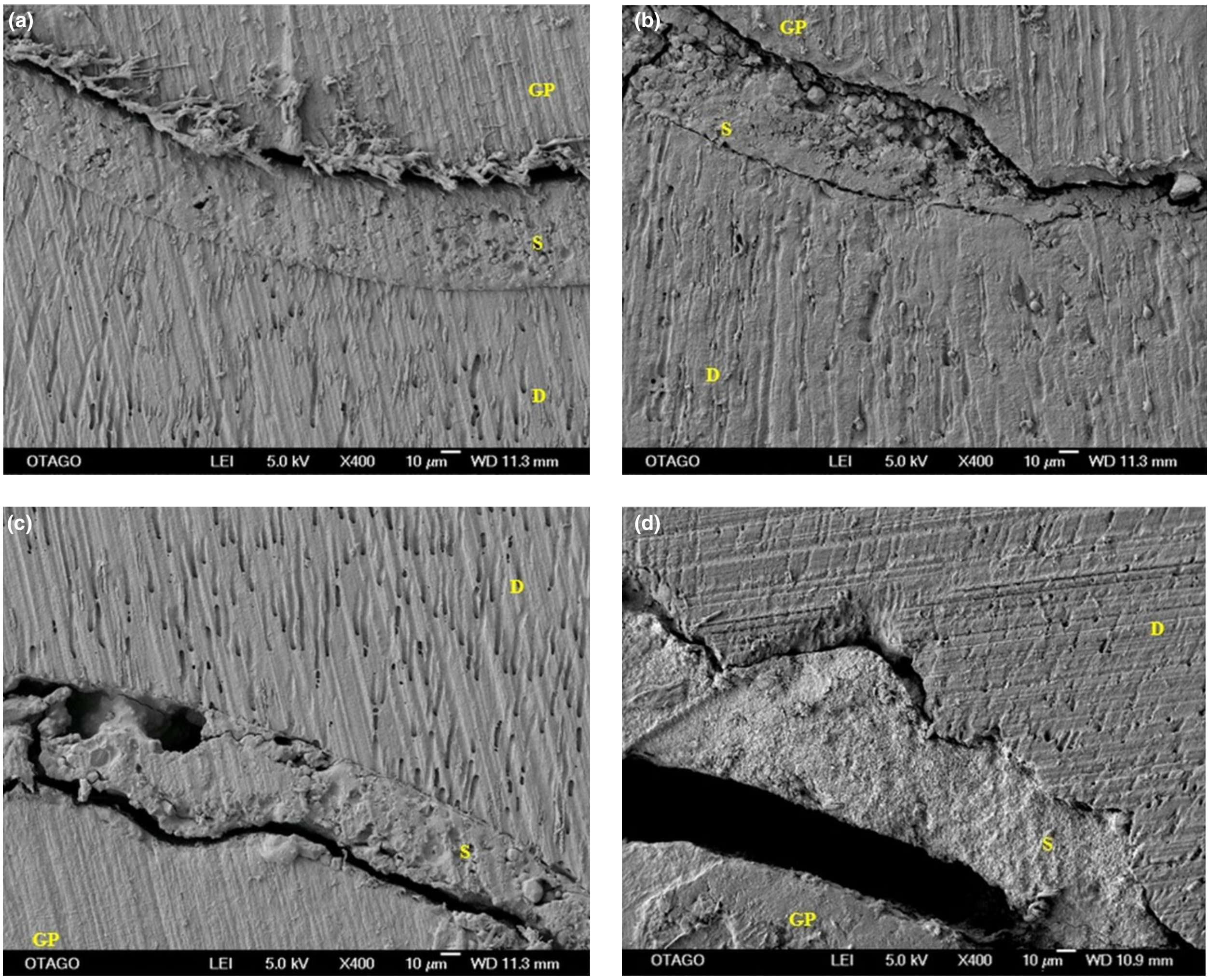

Figure 6 Representative SEM micrographs (×400) of sealer-dentine interface where GP is gutta percha, S is sealer and D is dentine. (a) good adaptation, (b) reasonable adaptation, (c) poor adaptation and (d) absent (no adaptation).

Table 4 Quality of adaptation of obturation material in mesio-distal and bucco-lingual aspects of teeth with and without the butterfly effect at coronal and mid-root levels (SEM)

			Adaptation quality (percentage)				
Root level	Butterfly effect	Direction	Good	Reasonable	Poor	Absent	Total
Coronal	Butterfly	Bucco-lingual	11	9	6		100
		Mesio-distal	10	10	4		
	Non-butterfly	Bucco-lingual	11	6	5		
		Mesio-distal	12	9	6	1	
Middle	Butterfly	Bucco-lingual	5	9	9	3	100
		Mesio-distal	6	8	9	1	
	Non-butterfly	Bucco-lingual	6	10	9	3	
		Mesio-distal	5	8	9		

powder/liquid or paste/paste ratio of the mixed material. Even small alterations to this ratio may cause a change in thickness and flow of the material. It is important for manufacturers to provide measuring equipment for clinicians to achieve ideal powder/liquid or paste/paste ratio of root canal sealers (21). In the present study, most of the sealers (EndoRez, AH Plus, Fillapex MTA) were in automix syringes, providing

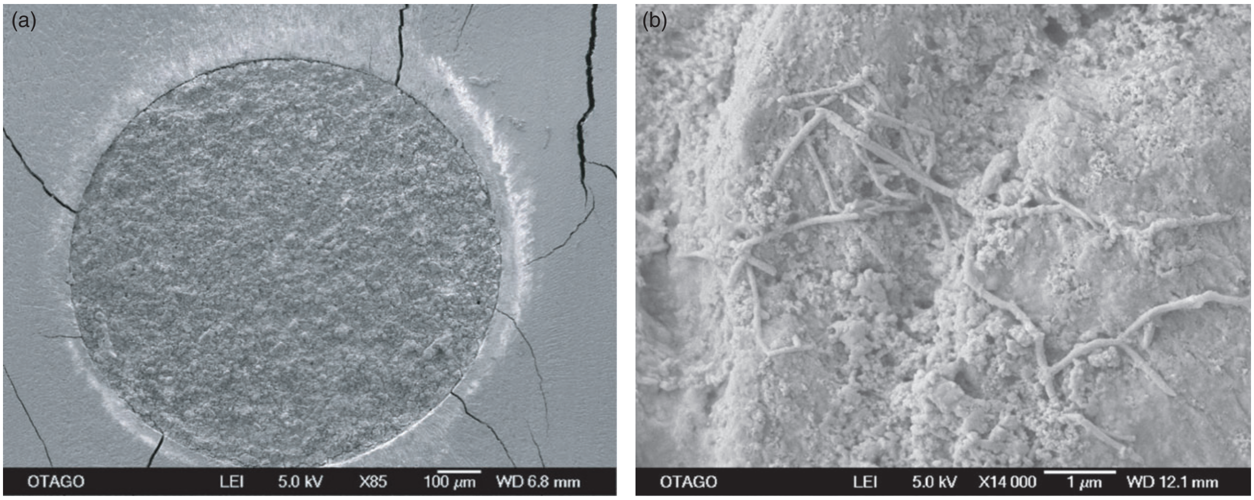

Figure 7 (a) Bird's eye-view SEM micrograph (×85) of the ProRoot MTA-dentine interface showing good adaptation. (b) High magnification SEM micrograph (×14 000) of ProRoot MTA within a dentinal tubule showing crystal-like formation.

standardised mixtures. Other materials (Pulp Canal Sealer and ProRootMTA) were mixed according to the manufacturer's instructions.

In this study the same filling method was used for all sealers. The literature provides no standard guidelines regarding techniques for different sealers. For example, one study reports that Fillapex MTA had significantly greater tubule penetration with a warm vertical technique vs. the single cone technique (22). On the other hand, another study reports that Fillapex MTA had superior penetration with cold lateral compaction (23), whereas another suggests that calcium silicate sealers showed inferior bond strength when the continuous wave technique was used (24). The manufacturers' recommendations are that calcium-silicate based sealers should be used with single cone obturation (25). The optimal obturation technique for different sealers remains controversial, but the technique performed in the current study was suitable for use with all the sealers tested.

This study reports that sealers and ProRoot MTA have superior adaptation and penetration in coronal sections of root compared with middle sections. This finding is in agreement with previous studies that investigated a variety of sealers and obturation techniques and have reported that the mean penetration is greater coronally (11,16,22,23,26). Although apical root sections were not included in the present study, regional differences may be explained by the increasing complexity of root canal anatomy and the reduced number and patency of dentinal tubules towards the apical portion of the root canal (14).

Another possible explanation for the differences is the use of the single cone obturation technique. As the GP cone is inserted, sealer may become displaced and air may become entrapped forming voids (27). Furthermore, greater compressive forces during obturation may have been applied coronally which would improve adaptation to the canal wall.

Studies have applied different microscopy techniques to investigate sealer penetration, such as SEM (11,28,29), light microscopy (30) and CLSM (16,22,23,25,26). There are no studies specifically comparing the accuracy of SEM and CLSM in determining depth of penetration and adaptation of root canal sealers.

In the present study, SEM offered a number of advantages for assessing the adaptation of sealers and ProRoot MTA. It allowed high magnification observation of the dentine-sealer or ProRoot MTA interface and detailed visualisation of the dentinal tubules and their contents (16) which cannot be achieved with other techniques. The SEM micrographs are easy to interpret and in this study, substantial inter-examiner agreement was achieved when analysing quality of adaptation.

On the other hand, SEM has limited use when measuring the depth of penetration. Preparation of root sections for SEM requires samples to be desiccated, highly polished and contain no surface smear layer. This can lead to loss of the sealer or ProRoot MTA from the dentine surface and thus an under representation of penetration depth. This may explain the seemingly low reported depths of penetration in some SEM studies (10,11,28,29).

To overcome these limitations of SEM, CLSM was used to accurately measure sealer and ProRoot MTA penetration. CLSM allows the observation of sealer penetration below the surface of the dentine, eliminating the need for destructive specimen preparation or smear removal which may result in loss of sealer. To facilitate fluorescence under confocal microscopy, the sealers and ProRoot MTA were labelled with rhodamine B dye. Rhodamine B has been used successfully in many studies as an indicator for sealer penetration (16,22,25). However, the preferential use of Fluro-6 over rhodamine B to label calcium-silicate based sealers has been suggested as rhodamine B is said to have a low affinity for calcium in such sealers (12). Although the use of rhodamine B did not seem to affect the fluorescence of any of the sealers investigated, Fluor-6 could be used in future studies to label Fillapex MTA and ProRoot MTA.

Conclusions

This study is the first to use CLSM and SEM to evaluate sealer and ProRoot MTA penetration into dentinal tubules of teeth with the butterfly effect. It highlights a potential weakness of some studies that have overlooked the effect as a confounder. Further research is warranted to investigate the clinical implications of teeth with the butterfly effect.

Within the limitations of this study, it can be concluded that:

- The butterfly effect influences sealer and ProRoot MTA penetration and adaptation in root canals. Roots with the effect have greater penetration bucco-lingually. This may enhance entombment of bacteria, which could lead to improved treatment outcomes.
- Coronal sections of roots have superior adaptation and penetration compared with middle sections. Penetration in middle sections was significantly more favourable in teeth without the butterfly effect.

Acknowledgements

We thank Miss Liz Girvan of the Otago Centre for Electron Microscopy and Mr Andrew Mc Naughton, University of Otago, for their time, guidance and knowledge. This research was supported by a University of Otago Fuller Scholarship.

Authors' contributions

The authors declare that they have contributed significantly to this study and are in agreement with the contents of this manuscript.

Disclosure statement

The authors deny any conflict of interest related to this study.

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