doi:10.1111/j.1365-2591.2008.01462.x

Microscopic appearance and apical seal of root canals filled with gutta-percha and ProRoot Endo Sealer after immersion in a phosphate-containing fluid

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Abstract

Weller RN, Tay KCY, Garrett LV, Mai S, Primus CM, Gutmann JL, Pashley DH, Tay FR. Microscopic appearance and apical seal of root canals filled with gutta-percha and ProRoot Endo Sealer after immersion in a phosphate-containing fluid. *International Endodontic Journal*, **41**, 977–986, 2008.

Aim To investigate the sealing quality of ProRoot Endo Sealer, a calcium silicate-based sealer and its morphologic characteristics after immersion in a phosphate-containing fluid (PCF).

Methodology Single-rooted canals were filled with gutta-percha and either ProRoot Endo Sealer or two commercially available zinc oxide eugenol (ZOE)-based and epoxy resin-based sealers. The sealers were allowed to set for 6 days and the filled teeth were immersed in PCF for 24 h before fluid leakage evaluation. After initial leakage evaluation at the 7th day, each filled root was restored and reimmersed in PCF for 28 days before the second phase of leakage evaluation at 35 days. Cryofractured specimens of additional teeth filled with the three sealers were examined using scanning electron microscopy after immersion in PCF for the two periods.

Results One-way repeated measures ANOVA and Tukey test revealed significant differences between the ZOE-based sealer at 35 days and the calcium silicate-based sealer at 35 days ($P < 0.001$), and between the ZOE-based sealer at 7 days and the calcium silicate-based sealer at 35 days ($P = 0.001$). No difference was found between the epoxy resin-based sealer and the calcium silicate-based sealer after both storage periods. Cryofractured calcium silicate-based sealer specimens demonstrated apatite-like crystalline deposits along the apical and middle thirds of the canal walls via transformation from amorphous calcium phosphate-like precursors.

Conclusions ProRoot Endo Sealer is comparable in sealing quality to the epoxy resin-based sealer and seals better than the ZOE-based sealer after immersion in PCF. The calcium silicate-based sealer also demonstrates *ex vivo* bioactivity when it comes into contact with phosphate ions.

Keywords: calcium silicate-based sealer, Flodec, fluid leakage, *in vitro* bioactivity.

Received 21 January 2008; accepted 28 June 2008

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Introduction

Calcium silicate-containing ProRoot MTA root canal repair material (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) has been used successfully as a root-end filling, perforation repair, apexification and capping material for vital pulp therapy (Torabinejad & Chivian 1999, Camilleri & Pitt Ford 2006, Roberts *et al.* 2008) because of its ability to stimulate cementogenesis and dentinogenesis (Torabinejad *et al.* 1997, Tziafas *et al.* 2002, Thomson *et al.* 2003, Apaydin *et al.* 2004, Baek *et al.* 2005, Tani-Ishii *et al.* 2007, Tomson *et al.* 2007). The material is bioactive as a result of the slow release of calcium and hydroxyl ions of its Portland cement component (Bentz & Garboczi 1992, Duarte *et al.* 2003). These ions interact with phosphate-containing fluids to form crystalline apatites (Camilleri *et al.* 2005, Sarkar *et al.* 2005, Bozeman *et al.* 2006, Tingey *et al.* 2008) via an amorphous calcium phosphate precursor phase (Tay *et al.* 2007).

Although ProRoot MTA has been advocated for case-selective orthograde root canal fillings (Hayashi *et al.* 2004, Vizgirda *et al.* 2004, Al-Hezaimi *et al.* 2005, Martin *et al.* 2007), the hardness of the set cement renders nonsurgical retreatment extremely difficult. Conversely, application of a calcium silicate-based sealer with cold lateral/warm vertical compaction techniques with gutta-percha as a filling material would create a bioactive/antimicrobial (Al-Hezaimi *et al.* 2006) sealer-dentine interface without compromising the retreatability of the filled canals. Whilst such a sealer would be advantageous for general purpose root fillings, it would be highly desirable in clinical situations such as root perforations or invasive cervical resorption (Heithersay 2004) in which repair with a calcium silicate-based cement and the subsequent filling of the root canal may be accomplished simultaneously using the same sealer that is mixed to different consistencies.

ProRoot Endo Sealer (Dentsply Tulsa Dental Specialties) is a calcium silicate-based endodontic sealer that is designed to be used in conjunction with a root filling material in either the cold lateral, warm vertical or carrier-based filling techniques. The major components of the powder component are tricalcium silicate and dicalcium silicate, with the inclusion of calcium sulphate as a setting retardant, bismuth oxide as a radiopacifier and a small amount of tricalcium aluminate. The liquid component consists of a viscous aqueous solution of a water-soluble polymer. The objectives of this study were to investigate the sealing ability of root canals that were filled with gutta-percha and the calcium silicate-based sealer and to examine the morphologic characteristics of the set material after immersion in a phosphate-containing fluid (PCF). The null hypothesis tested was that there is no difference in fluid leakage amongst the calcium silicate-based sealer and two commercially available, zinc oxide eugenol-based and epoxy resin-based root canal sealers.

Materials and methods

Sixty-six extracted human maxillary anterior teeth were stored in 0.9% sodium chloride containing 0.02% sodium azide (NaN_3) to prevent bacterial growth. These teeth were collected after patients' informed consents were obtained under a protocol reviewed and approved by the Human Assurance Committee of the Medical College of Georgia, USA. The experimental design consisted of three experimental groups ($n = 10$) for fluid leakage evaluation. Three teeth were used for each of the positive and negative control groups. Thirty additional teeth, randomly divided into three groups of 10 teeth each, were employed for morphologic characterization.

Cleaning, shaping and filling of the root canals were performed under an operating microscope (OPMI pico; Carl Zeiss Surgical Inc, Thornwood, NY, USA). Each experimental group contained 30% round canals and 70% oval-shaped canals, as determined by the use of buccolingual and mesiodistal radiographs. Canal patency was achieved with a size 15 Flex-o-file (Dentsply Maillefer, Ballaigues, Switzerland). Instrumentation was performed to 0.5 mm short of the radiographic apex with a crown-down technique using ProTaper nickel titanium rotary instruments (Dentsply Tulsa Dental Specialties) to size F4. The root canals were irrigated with 6.15% NaOCl between instrumentation, and with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) under passive ultrasonic irrigation (van der Sluis *et al.* 2007) for 1 min to remove canal wall smear layers.

Each debrided canal was dried with multiple paper points and trial fitted with a F4 gutta-percha master cone with tug-back. The three sealers evaluated for fluid leakage and morphologic examination were Pulp Canal Sealer (SybronEndo, Orange, CA, USA), AH Plus (Dentsply Caulk, Milford, DE, USA) and ProRoot Endo Sealer. The former two sealers were mixed according to the manufacturers' instructions. The calcium silicate-based sealer was mixed with a liquid-to-powder ratio of 1 : 2 and covered with moist gauze to avoid evaporation of the water component. Each canal was filled with a warm vertical compaction technique using a System B heat source (SybronEndo), backfilled with gutta-percha using a Calamus unit (Dentsply Tulsa Dental Specialties) and restored with Cavit (3M ESPE, St Paul, MN, USA). The teeth were stored at 100% relative humidity for 6 days to allow complete setting of the sealers and then immersed in a PCF (0.17 g KH_2PO4, 1.18 g Na_2HPO_4, 8.0 g NaCl, 0.2 g KCl in 1 L of deionized water containing 0.2 g of NaN_3 to prevent bacterial growth; pH 7.3) (Sarkar *et al.* 2005) for 24 h prior to leakage evaluation.

For the positive control, each canal was cleaned and shaped in the manner previously described. A master cone was inserted to within 0.5 mm of the working length with tug-back without the use of a sealer. The gutta-percha was seared off from the canal orifice with the System B heat source. For the negative control, the canal was cleaned and shaped and filled with gutta-percha and AH Plus sealer. After filling, the entire root was dipped into molten sticky wax to seal the root surface and apex.

Leakage evaluation

The filled teeth were decoronated using a slow-speed Isomet saw (Buehler Ltd, Lake Bluff, IL, USA) under water-cooling to obtain 17 mm long root segments. Leakage was evaluated using a fluid filtration design (Derkson *et al.* 1986) upgraded with a Flodec measuring device (De Marco Engineering, Geneva, Switzerland) that permits digital data collection at 1.04-s intervals (Monticelli *et al.* 2007). The Cavit was removed and the root segment was attached via its coronal orifice to an 18-gauge needle-perforated Plexiglas platform and sealed with cyanoacrylate glue. The external root surface was also covered with cyanoacrylate to within 2 mm of the apical foramen. Nitrogen gas pressure was applied at 10 psi (69 kPa) via a polyethylene tubing through the coronal end of the root segment. Fluid flow was recorded by monitoring the displacement of a water bubble inside the glass capillary tube of the Flodec device. Data were recorded as fluid flow over time and expressed as mean fluid flow ($\mu L\ min^{-1}$). After the initial fluid leakage evaluation, the root segment was carefully removed from the Plexiglas platform. The coronal orifice was restored with Cavit and the root segment was immersed in the PCF for an additional 28 days prior to the second period of fluid leakage evaluation at 35 days.

A $\log_{10}$-transformation of the data was performed to normalize the data before statistical evaluation. As the normality (Kolmogorow–Smirnoff test) and homoscedasticity (Levene test) assumptions of the transformed data appeared to be valid, they were analysed using one-way repeated measures ANOVA and Tukey multiple comparison test at $\alpha = 0.05$.

Morphologic examination

The remaining 30 teeth were cleaned, shaped and filled with gutta-percha and the respective sealer in the manner previously described and stored in PCF for the same two periods (i.e. five teeth were examined for each of the three sealers for each storage period; $5 \times 3 \times 2 = 30$). At the end of each storage period, longitudinal slits were made in the teeth, which were then cryofractured in liquid nitrogen. The cryofractured specimens were air-dried and sputtered-coated with gold/palladium for examination with a field emission-scanning electron microscope (FE-SEM; Model XL-30 FEG; Philips, Eindhoven, the Netherlands) at 5 keV.

Results

Statistical analysis of the fluid leakage results (Fig. 1) revealed highly significant differences amongst the three sealer groups examined after the two storage periods ($P < 0.001$). The power of the analysis with

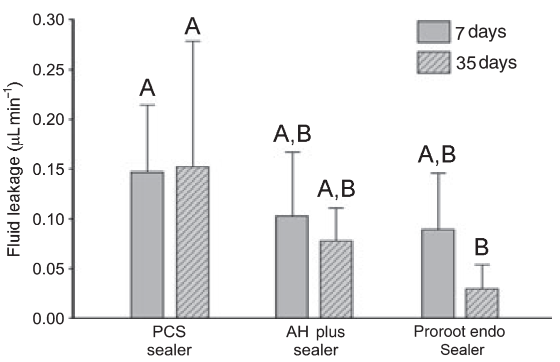

Figure 1 Bar chart depicting the fluid leakage results of the three groups of sealers after allowing to set completely at 100% relative humidity for 6 days and immersing in a phosphate-containing fluid for 24 h (i.e. after 7 days) or an additional 28 days (i.e. after 35 days). The median [range] for the Pulp Canal Sealer at 7 days (PCS-7), Pulp Canal Sealer at 35 days (PCS-35), AH Plus sealer at 7 days (AHP-7), AH Plus sealer at 35 days (AHP-35), ProRoot Endo Sealer at 7 days (EXP-7) and ProRoot Endo Sealer at 35 days (EXP-35) are (in $\mu L\ min^{-1}$): 0.129 [0.199], 0.093 [0.362], 0.096 [0.195], 0.082 [0.115], 0.058 [0.151] and 0.028 [0.078], respectively. In the bar chart, groups with the same letter above the columns denote no statistical significance ($P > 0.05$).

$\alpha = 0.05$ was 0.964. Of the 15 pairwise comparisons (Tukey test), only two comparisons exhibited significant differences: between Pulp Canal Sealer at 35 days and ProRoot Endo Sealer at 35 days ($P < 0.001$), and between Pulp Canal Sealer at 7 days and the ProRoot Endo Sealer at 35 days ($P = 0.001$). There was no fluid leakage in the negative control group. Leakage in the positive control group (48.53 ± 5.18 mL min^{-1}) was five orders of magnitude higher than the leakage exhibited by the three sealer groups.

Representative micrographs taken from the coronal, middle and apical thirds of the five ProRoot Endo Sealer-filled canals and examined after the initial 7-day storage period are shown in Fig. 2. The sealer layer was generally 20–25 μm thick (e.g. Fig. 2a – coronal third; Fig. 2c – apical third). Minimal sealer tags were seen in the smear layer-depleted radicular dentine (Fig. 2a – coronal third). The same feature could also be identified from the middle third of the canal walls (not shown). In the apical third of the canal walls, dentinal tubules were frequently absent or highly irregular in appearance, with minimal sealer penetration (Fig. 2c – apical third). In the coronal and middle thirds of the canal walls in which warm vertical compaction had been used, gutta-percha was sometimes in direct contact with the radicular dentine, producing short gutta-percha tags that displaced the smaller sealer particles into the tubules (Fig. 2b – middle third). In all the five specimens examined, submicron spherical bodies of variable dimensions (200 nm–0.7 μm in diameter) could be identified from the apical third of the canal walls. These spherical bodies were seen along the dentine surface (Fig. 2d – apical third) as well as the surfaces of the sealer particles (Fig. 2e – apical third). Unlike cocci bacteria, these spherical bodies appeared as aggregates of different sizes and did not exhibit a partially collapsed surface when examined after specimen desiccation and inside a high-vacuum environment.

Representative micrographs taken from the coronal, middle and apical thirds of the five ProRoot Endo Sealer-filled canals and examined after the 35-day storage period are shown in Fig. 3. In all the specimens, the sealer morphology in the coronal third of the canal walls (Fig. 3a – coronal third) was similar to that identified after the 7-day storage period. In these specimens, reaction products that were not previously observed from the 7-day storage specimens could always be seen within the porous sealer and along the sealer–dentine interface from the apical third (five or five), and frequently in the middle third of the canal walls (four of five). They consisted of cancellous appositions along the surfaces of sealer particles and needle-shaped crystalline clusters (Fig. 3b – middle third). These features are shown in higher magnification in Fig. 3c (middle third), wherein a sealer particle could be observed within the peripheral cancellous appositions. Along the apical third of the canal walls, some of the calcium silicate-based sealer particles appeared to have been modified, resulting in hollow spaces that were surrounded by a peripheral layer of the cancellous appositions (Fig. 3d – apical third). Needle-shaped crystalline clusters were also observed along the exposed sealer surface, adjacent to the sealer particles. This is better depicted in the higher magnification view (Fig. 3e – apical third), in which additional needle-shaped crystallites could also be observed within the sealer layer.

Figure 4a is a representative example of the AH Plus–dentine interface from a specimen taken after 35-day of immersion in the PCF. The sealer layer was about 20 μm thick. Numerous multifaceted fillers that are characteristic of AH Plus (Sevimay & Kalayci 2005) could be seen within the epoxy resin matrix. Figure 4b is a representative example of the Pulp Canal Sealer–dentine interface from a specimen taken after 35-day of immersion in the PCF. The sealer layer was about 15 μm thick. In these two sealer groups, gutta-percha was sometimes found to be in direct contact with dentine (not shown). Both the cancellous appositions and needle-shaped crystalline clusters were absent from cryofractured specimens derived from the AH Plus and Pulp Canal Sealer groups.

Discussion

Induction of apatite formation appears to be a common characteristic for silicate-containing biomaterials, as demonstrated by the *in vitro* bioactivity of tricalcium silicate (Zhao *et al.* 2005) and dicalcium silicate bone cements (Gou *et al.* 2005). ProRoot Endo Sealer depends on water for setting in a manner that is analogous to ProRoot MTA or MTA-Angelus products (Song *et al.* 2006, Camilleri 2007, Oliveira *et al.* 2007). Despite differences in reactivity, the hydration products of tricalcium silicate and dicalcium silicate consists of amorphous calcium silicate hydrate and calcium hydroxide (Birchall *et al.* 1978). Calcium hydroxide is released from the surface as the calcium silicates form hydrates (Goñi *et al.* 1996, Haga *et al.* 2005) and provides the source of calcium and hydroxyl ions for the precipitation of calcium phosphate phases in the

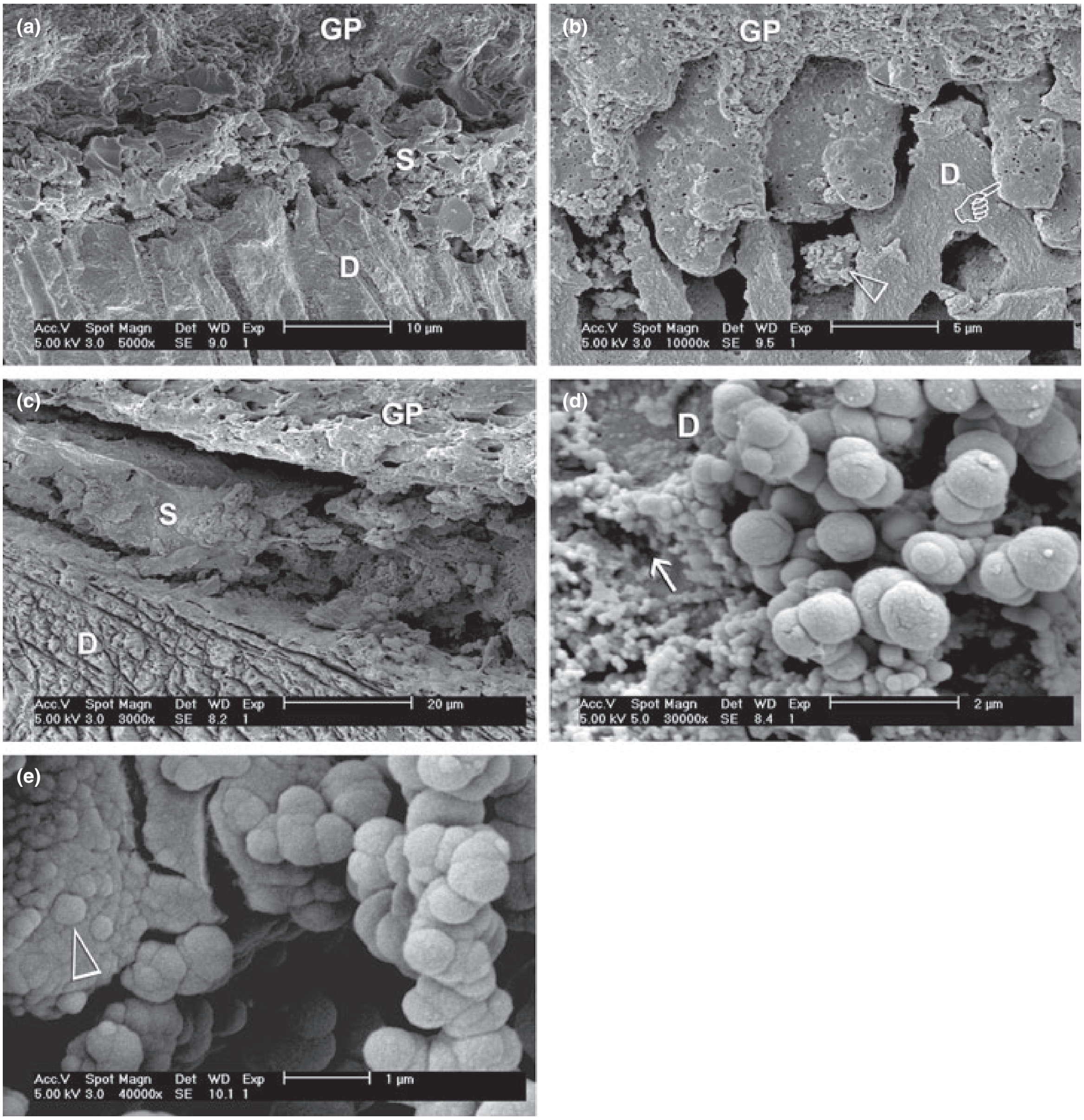

Figure 2 FE-SEM micrographs of cryofractured root canals filled with ProRoot Endo Sealer that has set for 6 days prior to immersing in a phosphate-containing fluid for 24 h (i.e. 7-day storage). S, sealer; D, radicular dentine; GP, gutta-percha. (a) A representative example taken from the coronal third of a root canal where the sealer was 10–20 μm thick. Sealer tags were infrequently observed as most of the sealer particles were larger than 2–3 μm in diameter. (b) A micrograph taken from the middle third of a root canal showing direct contact of the gutta-percha with the smear layer-depleted dentine, resulting in short sealer tags consisting of gutta-percha (pointer) and smaller sealer particles (open arrowhead) that had been displaced into the tubules. (c) The root filling dentine interface taken from the apical third of a root canal. The sealer was 15–20 μm thick. The radicular dentine from this part of the canal space appeared highly irregular. (d) Submicron spherical bodies (open arrowhead) could be observed along the apical part of the root canal walls where the gutta-percha and sealer had been dislodged during cryofracture. These submicron globular bodies are probably amorphous calcium phosphate phases that were formed by the interaction of calcium ions released by the calcium silicates with the phosphate-containing fluid. Arrow: patent dentinal tubule. (e) A micrograph taken from the apical third of another root canal showing additional submicron spherical bodies along the surface of the sealer particles.

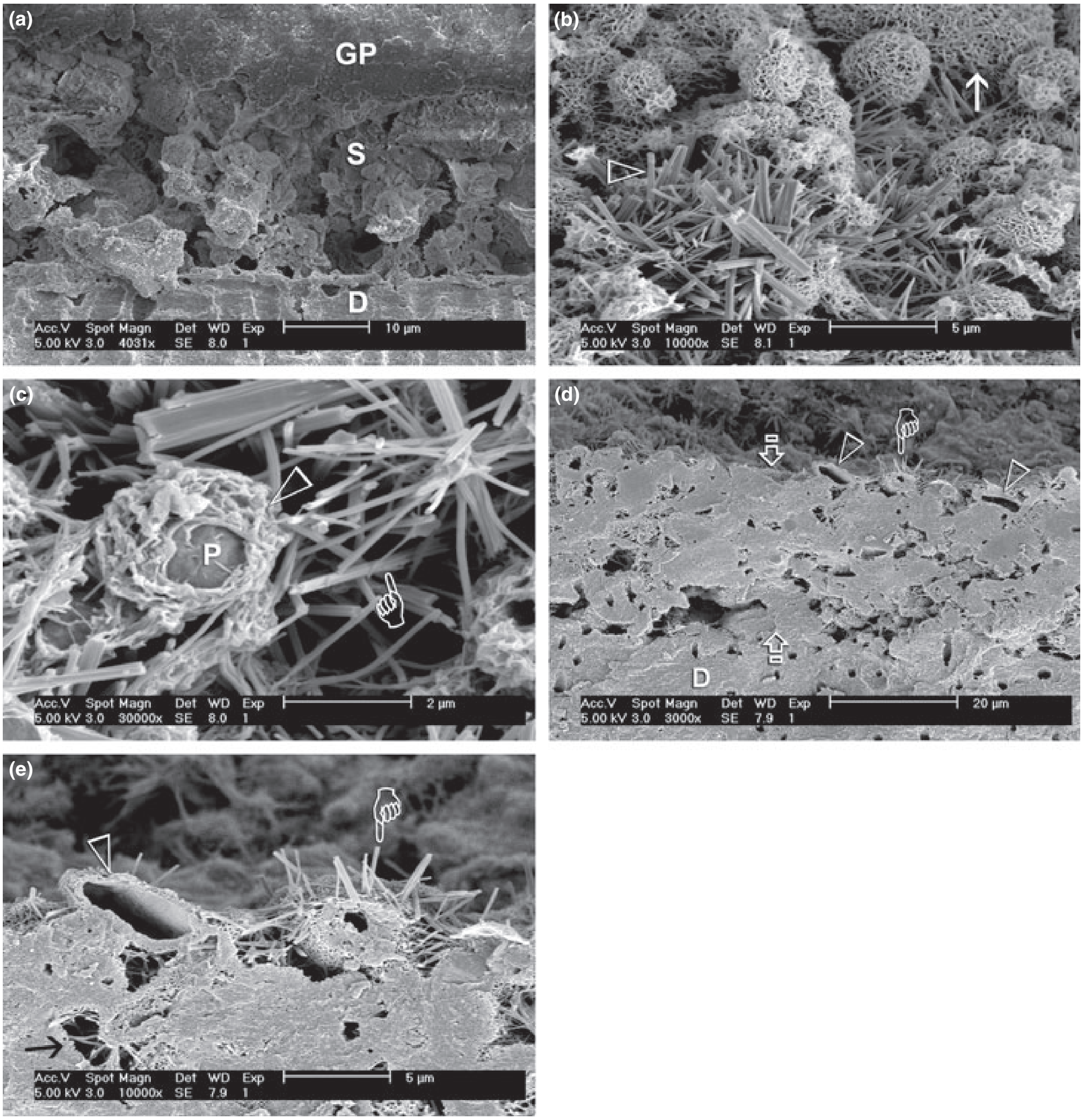

Figure 3 FE-SEM micrographs of cryofractured root canals filled with ProRoot Endo Sealer that has set for 6 days prior to immersing in a phosphate-containing fluid for 29 days (i.e. 35-day storage). S, sealer; D, radicular dentine; GP, gutta-percha. (a) A micrograph taken from the coronal third of a root canal. The sealer was 20 μm thick (between open arrows). There was no difference in the sealer morphology when compared with the 7-day specimens. (b) A micrograph taken from the middle third of a root canal in which the gutta-percha had been dislodged during cryofracture. The sealer particles were covered by a matrix consisting of hydration products (arrow). Needle-shaped crystalline clusters could also be seen (open arrowhead). (c) A high magnification view of the fractured sealer showing the presence of a matrix (open arrowhead) around a large sealer particle (P). The matrix probably represents the calcium-silicate-hydrate phase that is formed when tricalcium silicate and dicalcium silicate react with water. The adjacent needle-shaped crystals (pointer) probably represent apatite crystals that were transformed from the amorphous calcium phosphate phases shown in the previous figure. (d) A low magnification view of the apical third of a root canal with the gutta-percha dislodged, revealing a 20-mm thick sealer layer (between open arrows). Similar needle-shaped crystallite clusters could be identified along the sealer surface (pointer). Some of the sealer particles were rendered hollow after immersion in the phosphate-containing fluid and were surrounded by a reactionary matrix phase (open arrowheads). (e) A higher magnification view of Fig. 3d. Additional needle-shaped crystallites (arrow) could be seen within the porous sealer layer.

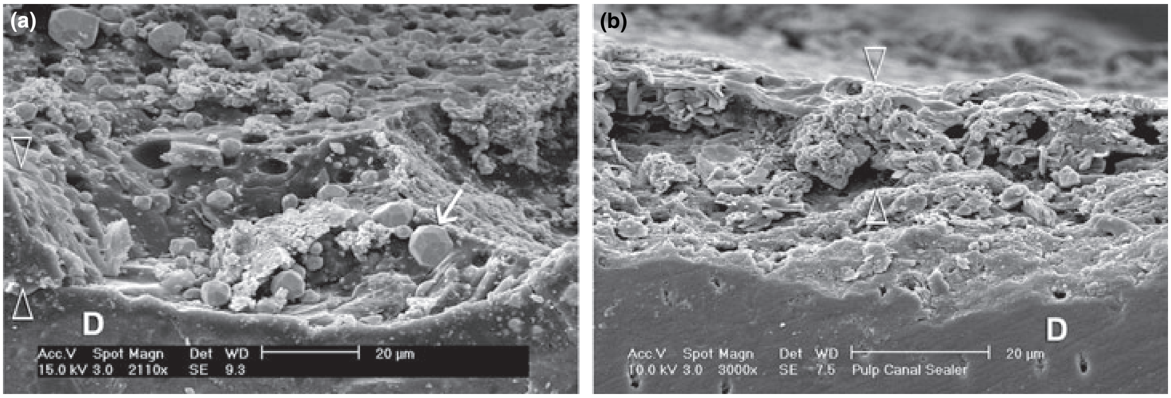

Figure 4 (a) FE-SEM micrograph of an AH Plus-filled, cryofractured root canal taken from the apical third of the canal wall after the 35-day storage period in the phosphate-containing fluid. The gutta-percha had been dislodged and a 20-μm thick layer of the resin-based sealer (between open arrowheads) could be seen attached to the radicular dentine (D). Multifaceted fillers (arrow), characteristic of the AH Plus filler, could be seen within the resinous matrix. There were no submicron spherical bodies or needle-shaped crystalline clusters similar to those identified from the ProRoot Endo sealer after immersion in the phosphate-containing fluid. (b) FE-SEM micrograph of a Pulp Canal Sealer-filled, cryofractured root canal taken from the apical third of the canal wall after the 35-day storage period in the phosphate-containing fluid. The gutta-percha had been dislodged and a 15-μm thick layer of the resin-based sealer (between open arrowheads) could be seen attached to the radicular dentine (D). No submicron spherical bodies or needle-shaped crystalline clusters similar to those identified from the ProRoot Endo sealer could be observed after immersion in the phosphate-containing fluid.

presence of the PCF. The amount of tricalcium aluminate has also been reduced in the calcium silicate-based sealer so that the amount of ettringite hydration products (Bailey & Hampson 1983) is probably to be minimal.

The fluid leakage results obtained after the two storage periods warrant rejection of the null hypothesis that there is no difference in fluid leakage amongst the calcium silicate-based sealer and the two commercially available root canal sealers. The relatively severe leakage observed particularly during the initial 7-day period might be caused by the inclusion of 70% oval-shaped canals. As the seal in these canals is inferior to those in round canals, the overall quality of seal for each experimental group could have been compromised (van der Sluis *et al.* 2005, De-Deus *et al.* 2008). It is also inappropriate to compare leakage results from different studies on the same endodontic sealer unless the percentages of oval-shaped canals utilized in these studies are known. During the initial 7-day storage period in which the specimens were immersed in PCF, submicron spherical bodies were observed that probably represented amorphous calcium phosphate (ACP) precursors (Eanes 2001) that are first formed when white Portland cement interacts with PCF (Tay *et al.* 2007). Diffusion of phosphate ions from the PCF into the microporous sealer via the apical foramen could have generated the ACP-like precursors. This probably explains why the submicron bodies were only observed initially along the apical third of canal walls. With more extended periods of PCF immersion, there would be more phosphate ions available for the interaction with the calcium and hydroxyl ions released by the calcium silicate-based sealer. This probably explains why reaction products could be seen up to the middle third of the canal walls at the 35-day examination period. During this period, it is probably that the ACP precursors had been transformed into calcium-deficient apatites (LeGeros 1991, Gadaleta *et al.* 1996, Eanes 2001), producing the needle-shaped crystal clusters shown in Fig. 3. As the ACP phases were depleted, the underlying calcium silicate hydrate hydration phase were exposed (Garrault *et al.* 2006), giving the appearance of a cancellous amorphous surface layer on the surface of the calcium silicate particles. No attempt was made to analyse the comparatively smooth ACP-like phases and the apatite-like crystalline clusters this study, as these phases were present either on the surface of the calcium phosphate-rich dentine and on the surface of the fractured sealer. The use of energy dispersive X-ray analysis to analyse these surface phases would have yielded information that includes the subsurface elemental composition of the dentine and sealer components. Likewise, these phases were not amendable for collection and purification for X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) analyses.

Although ProRoot Endo Sealer did not seal better than the two commercially available sealers initially (Fig. 1), the potential bioactive property of this calcium silicate-based sealer triggered apatite deposition as it came into prolonged contact with PCF. This resulted in filling of gaps and previously unfilled lateral canals with apatite clusters. Being porous, these crystalline clusters did not result in a statistically significant reduction in fluid leakage. However, the continuous release of calcium hydroxide and the ability of the calcium silicate-based sealer to modify its reaction products in response to the presence of phosphate ions may provide dynamic alkaline stresses that challenge the adaptability of planktonic bacterial aggregates and bacteria biofilms along filled root canal walls (Distel *et al.* 2002, Chávez de Paz 2007, Chávez de Paz *et al.* 2007). This hypothesis requires substantiation in future studies.

Because of the calcium-chelating property of EDTA, the use of this irrigant as the final rinse has been shown to disrupt the hydration of mineral trioxide aggregate, resulting in decreased hardness and less than optimal biocompatibility (Lee *et al.* 2007). A calcium and magnesium-free, 10 mmol L^{-1} phosphate-containing PCF was used instead of a simulated body fluid as it was anticipated that the PCF would be a potential final rinse prior to filling canals with the calcium silicate-based sealer. As calcium silicates require water for setting and maturation (Möser & Stark 2002), it may be advantageous to leave canals slightly moist prior to filling. It is interesting to observe that some of the sealer particles present in the coronal third of the canals appeared as discrete particles (Fig. 2d) that were not bound by a hydration phase. During warm vertical compaction, heat generated by the System B tip could have evaporated residual water that was present within tubular orifices, resulting in insufficient hydration of the sealer particles in this region. Thus, it can be speculated that the coronal seal of the experimental sealer may be improved by placing a water or PCF-moistened cotton pellet over the canal orifice prior to the placement of a provisional restoration. As a warm vertical compaction technique was the only method used to fill the teeth in this study, it would be interesting to see if apatite-like crystals can be identified from the coronal aspect of the canals when a cold lateral condensation technique is employed. These clinically relevant issues should be the subjects of investigation in future studies.

Conclusion

Within the limits of this study, it may be concluded that ProRoot Endo Sealer is comparable in sealing quality to a commercially available zinc oxide eugenol-based sealer and a commercially available epoxy resin-based sealer. The calcium silicate-based sealer also demonstrates *in vitro* bioactivity when it comes into contact with phosphate ions.

Acknowledgements

This study was supported by Dentsply Tulsa Dental Specialties. Dr Primus and Dr Gutmann served as consultants for Dentsply Tulsa Dental Specialties. The authors are grateful to Mrs Marie Churchville and Mrs Michelle Barnes for their secretarial support.

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