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Dynamic sealing ability of MTA root canal sealer

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Abstract

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Aims To evaluate (i) the sealing ability of two sealers, mineral trioxide aggregate sealer (MTAS) and Pulp Canal Sealer (PCS), used with gutta-percha utilizing the fluid filtration method, (ii) leaching and surface characteristics in Hank's balanced salt solution (HBSS) over a period of time.

Methodology Surface characteristics in HBSS were evaluated under the scanning electron microscope after 1 and 28 days, and the leaching of both sealers were assessed by inductively coupled plasma atomic absorption spectrometry (ICP-AAS). In addition, 24 single rooted extracted teeth were root filled using warm vertical compaction with either MTAS or PCS used as sealers with gutta-percha. Four teeth were used as positive and negative controls. Sealing ability was evaluated after 1 or 28 days using the fluid filtration method.

Results Mineral trioxide aggregate sealer exhibited crystalline deposits rich in calcium and phosphorus on its surface when in contact with a physiological solution. These crystalline deposits were absent in PCS and on MTAS stored at 100% humidity. The sealing ability of MTAS was similar to that of PCS.

Conclusions The novel sealer based on mineral trioxide aggregate had comparable sealing ability to a proprietary brand sealer cement. In contact with a simulated body fluid, the MTA sealer released calcium ions in solution that encouraged the deposition of calcium phosphate crystals.

Keywords: endodontic sealer, fluid filtration, inductively coupled plasma, mineral trioxide aggregate, Portland cement, Pulp Canal Sealer, scanning electron microscopy.

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Introduction

Mineral trioxide aggregate (MTA) is used primarily to seal lateral root perforations (Lee *et al.* 1993, Pitt Ford *et al.* 1995) and as a root-end filling material (Torabinejad *et al.* 1995, 1997, Chong *et al.* 2003, Saunders 2008). MTA can also be used for a variety of other applications (Schwartz *et al.*1999, Torabinejad & Chivian 1999) including pulp capping (Pitt Ford *et al.* 1996, Bakland 2000, Aeinehchi *et al.* 2003, Faraco Junior & Holland 2004) and as a dressing over pulpotomies in permanent teeth (Holland *et al.* 2001) and during apexification procedures (Witherspoon *et al.* 2008). It is a bioactive material that produces calcium hydroxide (Camilleri 2007, 2008a), which is released in solution (Fridland & Rosado 2003, Tanomaru-Filho *et al.* 2009) and induces formation of hydroxyapatite structures in simulated body fluid (Sarkar *et al.* 2005, Bozeman *et al.* 2006). Newer developments of MTA include its use as a root canal sealer. Currently, three MTA sealer formulations are available; Endo-CPM-Sealer (EGEO srl, Buenos Aires, Argentina), MTA Obtura (Angelus, Soluções Odontológicas, Londrina PR, Brazil) and ProRoot Endo Sealer (Dentsply Maillefer, Ballaigues, Switzerland). The composition of CPM sealer after mixing is reported to be 50% MTA (SiO_2, K_2O, Al_2O_3, SO_3, CaO and Bi_2O_3), 7% SiO_2, 10% $CaCO_3$, 10% Bi_2O_3, 10% $BaSO_4$,

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1% propylene glycol alginate, 1% propylene glycol, 1% sodium citrate and 10% calcium chloride (Gomes-Filho *et al.* 2009). MTA-Obtura is a mixture of white MTA with a proprietary viscous liquid (Monteiro Bramante *et al.* 2008). ProRoot Endo Sealer is calcium silicate–based endodontic sealer. The major components of the powder of ProRoot Endo Sealer are tricalcium silicate and dicalcium silicate, with inclusion of calcium sulphate as setting retardant, bismuth oxide as radiopacifier and a small amount of tricalcium aluminate. Tricalcium aluminate is necessary for the initial hydration reaction of the cement. The liquid component consists of viscous aqueous solution of a water-soluble polymer (Weller *et al.* 2008, Huffman *et al.* 2009). The use of water-soluble polymers mixed with materials based on Portland cement added to the water to improve the workability has been reported (Camilleri *et al.* 2005a, Camilleri 2008b,c,d,e). The polymer did not seem to affect the biocompatibility of the materials (Camilleri *et al.* 2005a, Camilleri 2008e), and the hydration characteristics were similar to those reported for MTA (Camilleri 2009).

Sealers based on MTA have been reported to be biocompatible, stimulate mineralization (Gomes-Filho *et al.* 2009), and encourage apatite-like crystalline deposits along the apical and middle thirds of canal walls (Weller *et al.* 2008). These materials exhibited higher push-out strengths than Pulp Canal Sealer (PCS) particularly after storage in simulated body fluid (Huffman *et al.* 2009) and had similar sealing properties to epoxy resin-based sealer when evaluated using the fluid filtration system (Weller *et al.* 2008).

Root canal sealers are used in conjunction with gutta-percha to fill root canals in various methods, namely cold lateral condensation, warm vertical compaction or carrier-based techniques. The function of the sealer is to obliterate discrepancies such as grooves and lateral depressions (Zielinski *et al.* 2008) that cannot be filled with gutta-percha, to improve the marginal adaptation to the dentinal walls (Cobankara *et al.* 2006) and to fill lateral canals (Venturi *et al.* 2005). The final root filling should prevent microleakage and bacterial contamination (Siqueira & Rocas 2007). Gutta-percha is impermeable; thus, any leakage occurs at the sealer to gutta-percha and sealer to tooth interfaces (Hovland & Dumsha 1985). Microleakage is routinely assessed by leakage to tracers, namely dyes (Beckham *et al.* 1993, Zaia *et al.* 2002), bacteria (Barthel *et al.* 1999) and endotoxin (Trope *et al.* 1995). Alternatively, the assessment can be performed using a fluid filtration device (Wu *et al.* 1998a, Bouillaguet *et al.* 2008). The fluid filtration method has already been used to evaluate the sealing ability of ProRoot MTA used for root-end filling with (Bates *et al.* 1996, Tang *et al.* 2002) and without (Pelliccioni *et al.* 2007) the use of water for hydration, furcation repair (Weldon *et al.* 2002, Hardy *et al.* 2004), and orthograde plugs (Martin *et al.* 2007). It has also been used for MTA Angelus (De-Deus *et al.* 2007) and MTA Bio, a laboratory-controlled water-based cement (De-Deus *et al.* 2007, 2008). The sealing ability of sealers based on MTA namely ProRoot Endo Sealer (Weller *et al.* 2008), other variants of MTA (Gandolfi *et al.* 2007) have also been evaluated with this method.

The aim of this study was to evaluate the sealing ability of two sealers, MTA sealer and PCS, used with gutta-percha utilizing the fluid filtration method as well as leaching and surface characteristics in Hank's balanced salt solution (HBSS) over a period of time.

Methodology

Materials used in this study included PCS (Kerr-Hawe S.A., Bioggio, Switzerland) and mineral trioxide aggregate sealer (MTAS). The MTAS consisted of a mixture of 80% white Portland cement (Aalborg white, Aalborg, Denmark) and 20% bismuth oxide (Fischer Scientific, Leicester, UK). The PCS was mixed according to manufacturer's instructions, whilst the MTAS was mixed in water to powder ratio of 0.30 with an addition of 20 $\mu L\ g^{-1}$ of cement of water soluble polymer (Degussa Construction Chemicals, Manchester, UK) added to the mixing water (Camilleri 2009). The materials were tested after immersion in a simulated body fluid namely Hank's Balanced salt solution (HBSS H6648; Sigma–Aldrich, Gillingham, UK). The composition of the HBSS was ($g\ L^{-1}$) 0.4 KCl, 0.06 KH_2PO_4 anhydrous, 0.35 $NaHCO_3$, 8.0 NaCl, 0.05 Na_2HPO_4 anhydrous and 1.0 D-glucose.

Scanning electron microscopy of the cements

Diskettes 10 mm in diameter and 1 mm high of PCS and MTAS were prepared. Half the diskettes were cured at 100% humidity and the other half were cured in HBSS for either 1 or 28 days. Surface morphology was evaluated after 1 and 28 days under the environmental scanning electron microscope (ESEM Zeiss EVO 50; Carl Zeiss, Oberkochen, Germany) and X-ray energy dispersive analysis (EDX) at an accelerating voltage of 20 kV was performed using a secondary electron detector (EDAX, Oxford INCA 350 EDS detector). The

elemental analysis (weight % and atomic %) of the specimens was performed applying ZAF correction method. The powders of each cement type were also analysed by EDX.

Evaluation of leaching

The chemical analysis of the cement products released into simulated body fluid was performed using inductively-coupled plasma atomic absorption spectroscopy (Varian Medical Systems, Palo Alto, CA, USA). The PCS and MTAS were mixed in a similar way as the previous experiments producing diskettes 10 mm in diameter and 1 mm high. Six diskettes for every material tested were prepared. The specimens were cured for 24 h at 37 °C and 100% humidity after which they were weighed to an accuracy of 0.0001 g and immersed in either 3 mL water or HBSS in closed sterile containers (Labplex, Birmingham, UK). The specimens were removed after 1, 14 and 28 days. Containers filled with water and HBSS were used as controls. The leachates were analysed for aluminium, bismuth, calcium, silicon, silver and zinc. The amount of leachate was calculated in $\mu g\ g^{-1}$ by using the following formula:

$$\text{Amount of leachate per weight of cement} = \frac{\text{amount of leachate per litre} \times 0.003 \times 1000}{\text{weight of cement pellet}}$$

Evaluation of sealing ability

Sample preparation

Twenty-four single-rooted teeth extracted for periodontal reasons were used. The study was performed on two experimental groups consisting of 10 teeth in each group. Four teeth were used as positive and negative controls. The teeth selected had similar dimension of root-apex namely a size 30 K-file, a similar diameter of root canal, no sclerotic dentine, circular cross-section of canal and no elliptic section, absence of caries and previous root fillings, absence of lateral canals and root curvature.

Radiographs were taken in the bucco-lingual and mesio-distal directions to establish the canal shape (circularity of cross-section) and to determine the presence of any root curvature and lateral canals. The teeth were decoronated, and the root length was standardized to 12 mm. The root canals were accessed, and canal patency was established using a size 15 K-file. Instrumentation was performed to 0.5 mm short of the radiographic apex using the crown-down technique with ProTaper rotary nickel-titanium instruments (Dentsply Maillefer). The apical preparation was performed to a size 30 master apical file. The diameter of root-apex was standardized in all samples with a size 30 K-file. The canal was irrigated with 10 mL of 5% NaOCl (Ogna, Milano, Italy) between instruments followed by 2 mL EDTA (Glyde file prep; Dentsply Maillefer, Montigny de Bretonneux, France) to remove the smear layer. The canals were dried with paper points and a master Gutta-percha cone (Dentsply Maillefer) was fitted to length and checked for tug-back. The canals were coated with the sealers under study using a size 20 reamer rotated anticlockwise. The master cone was coated with sealer and placed in the canal to working length. The cone was cut to the orifice and compacted with System B plugger (Sybron Endo, Orange, CA, USA) leaving a space of 2 mm coronally. Each root was radiographed to establish the adequacy of filling. An 18-gauge needle inserted across a plexiglass platform was introduced in the canal orifice and the root samples were attached to the platform with cyanoacrylate glue. The external root surface was coated with two layers of nail varnish (Paris, Bellure, Belgium) to seal the surfaces. For the positive control, the root canals of two teeth were cleaned and shaped as described earlier and filled with a master cone and no sealer. The negative control was prepared as described earlier, filled with gutta-percha and PCS and the apex was sealed off with bonding agent (Scotchbond; 3M ESPE, Milan, Italy) and nail varnish. The plexiglass support and tooth assembly was placed in 10 mL of HBSS keeping the free end of the needle un-immersed to mimic the clinical situation, where the coronal end of the tooth is not in contact with biological fluids. The samples were stored at 37 °C in sealed containers.

Leakage evaluation

The set-up was connected to a fluid conductive system working at a hydraulic pressure of 6.9 kPa to measure fluid movement (Fig. 1); the system used was as reported previously (Gandolfi *et al.* 2007, Pelliccioni *et al.* 2007) The fluid filtration rate was measured over three 4-min periods at 1-min intervals and the mean calculated. The results were expressed as $\mu L\ min^{-1}$. The following procedure was repeated after 1 and 28 days following root filling. The specimens were kept at 37 °C and the HBSS was changed weekly.

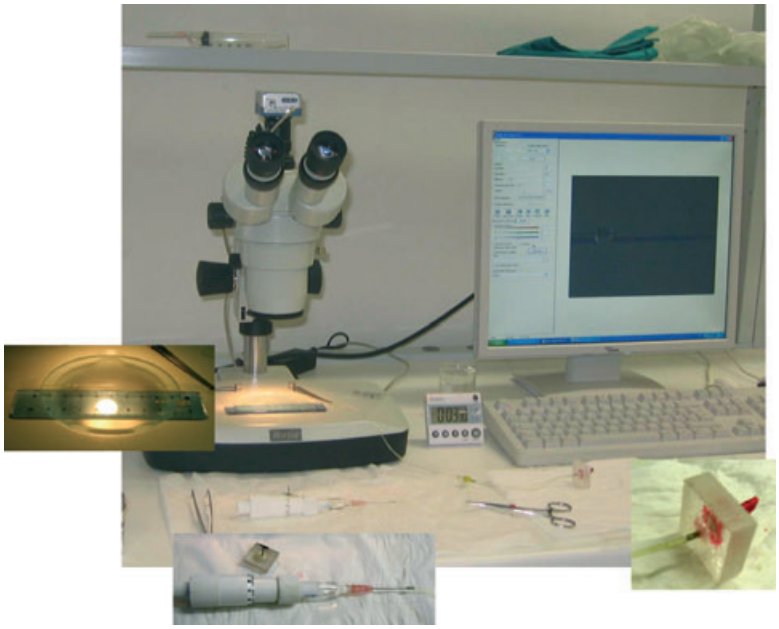

Figure 1 Set-up for determination of sealing ability using a fluid conductive system.

Statistical analysis

The data was evaluated using SPSS (Statistical Package for the Social Sciences) software (SPSS Inc., Chicago, IL, USA). Analysis of Variance (ANOVA) with $P = 0.05$ was used to perform multiple comparison tests.

Results

Scanning electron microscopy of the cements

The results for the scanning electron microscopy for both MTAS and PCS are shown in Fig. 2. The MTAS powder had a granular surface appearance with elongated bismuth oxide particles interspersed within the structure (Fig. 2A1). EDX analysis showed the material to be composed of calcium, silicon, aluminium and bismuth (Fig. 2A2). The PCS had a larger particle size distribution (Fig. 2A3) and was composed of zinc and silver (Fig. 2A4). Immersion of MTAS in HBSS resulted in a crystalline deposition over the cement surface after 7-day immersion (Fig. 2B3, B5) and also after 28-day immersion (Fig. 2C3) in HBSS. These crystalline deposits were not present in the MTAS cured at 100% humidity (Fig. 2B1, C1). The crystalline deposits were mainly composed of calcium and phosphorus (Fig. 2B4, B6) initially, with sodium and chlorine peaks at later curing times (Fig. 2C4). The pulp canal sealer surface demonstrated considerable porosity (Fig. 2B7, B9, C5, C7). The curing method did not affect the PCS surface and its chemical composition (Fig. 2B8, B10) at an early age. At 28 days, however, a chlorine peak was observed in the PCS cured at 100% humidity (Fig. 2C8).

Evaluation of leaching

The results for leaching of both sealers in water and in HBSS are shown in Table 1a,b, respectively. The levels of sodium and phosphorus ions in HBSS blank solution and the ions detected in both materials are shown in Table 2. MTAS leached a high level of calcium ions in both soaking solutions. The calcium ion release increased with time and the levels were higher in water than in HBSS. Bismuth was also released in solution with more bismuth being released in HBSS than in water. PCS leached zinc and silica in solution. This leaching was more marked in water. Both sealers had high levels of sodium when soaked in HBSS, but the levels of phosphorus were high for PCS but much lower and reducing to practically below detection limits at 28 days for MTAS.

Evaluation of sealing ability

The sealing ability of the two sealers evaluated using the fluid filtration method is shown in Fig. 3. There was no difference between the two sealers both at 7 days

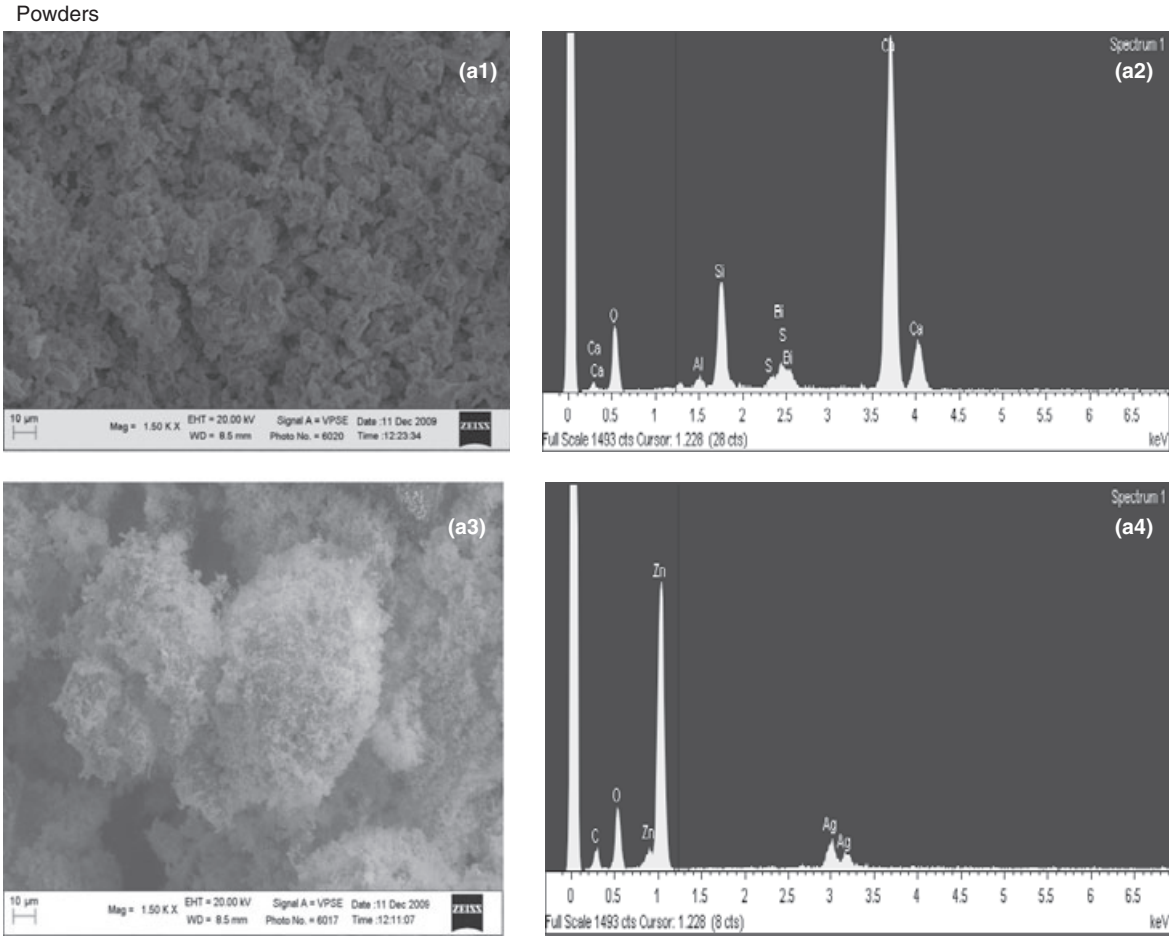

Figure 2 Scanning electron micrographs and X-ray energy dispersive analysis of A: powders (1,2): mineral trioxide aggregate sealer (MTAS), (3, 4): pulp canal sealer (PCS); B: materials cured for 7 days, (1–6): MTAS, (7–10): PCS, (1,2,7,8): cured at 100% humidity, (3–6,9,10): cured in Hank's balanced salt solution (HBSS); C: materials cured for 28 days, (1–4): MTAS, (5–8): PCS, (1,2,5,6): cured at 100% humidity, (3,4,7,8): cured in HBSS; (×1.5K magnification).

($P = 0.301$) and at 28 days ($P = 0.381$). The negative control exhibited little or no leakage, whilst the positive control demonstrated a high level of leakage that increased over the 28-day period.

Discussion

In this study, a new material based on MTA was investigated. This novel material was composed of Portland cement and bismuth oxide, which were mixed with water and a water-soluble polymer. PCS was used as control. This material was chosen because it had a powder and liquid formulation and has been used for a long time in clinical dentistry. The PCS powder was reported to be composed of 34% ZnO, 25% Ag, 30% resins, 11% thymol iodide and the liquid of Canada balsam and eugenol (Kerr data sheet). The peaks for zinc and silver were also demonstrated in this study. MTA powder is constituted of tricalcium and dicalcium silicate, tricalcium aluminate and bismuth oxide (Camilleri 2007, 2008a). Elemental composition of MTAS reported in this study is similar to the elemental composition published for ProRoot MTA (Camilleri *et al.* 2005b, Asgary *et al.* 2006). Pulp canal sealer was characterized by a porous structure as demonstrated by the environmental scanning electron microscope. Contact of PCS with a simulated body fluid had no effect on the surface characteristics of this material. On the other hand, a crystalline deposit consisting of calcium and phosphorus was present on the MTAS

7 day curing

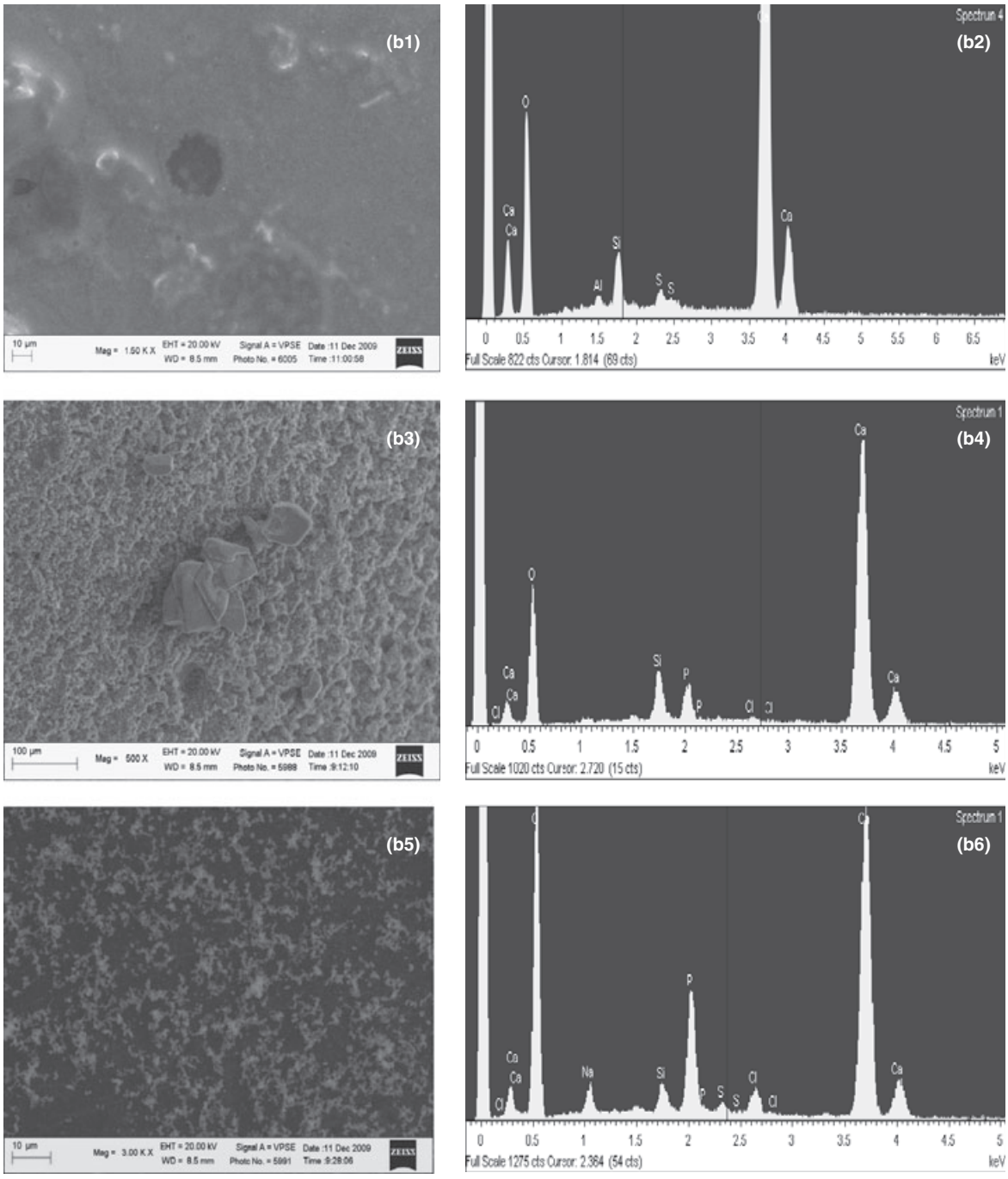

Figure 2b(1–6) (Continued).

surface when the MTA sealer was in contact with a simulated body fluid. MTAS released a high level of calcium ions in solution as indicated by the results of leaching. These calcium ions bind to the phosphates that are present within the simulated body fluid. This again was verified from the ICP results, where the

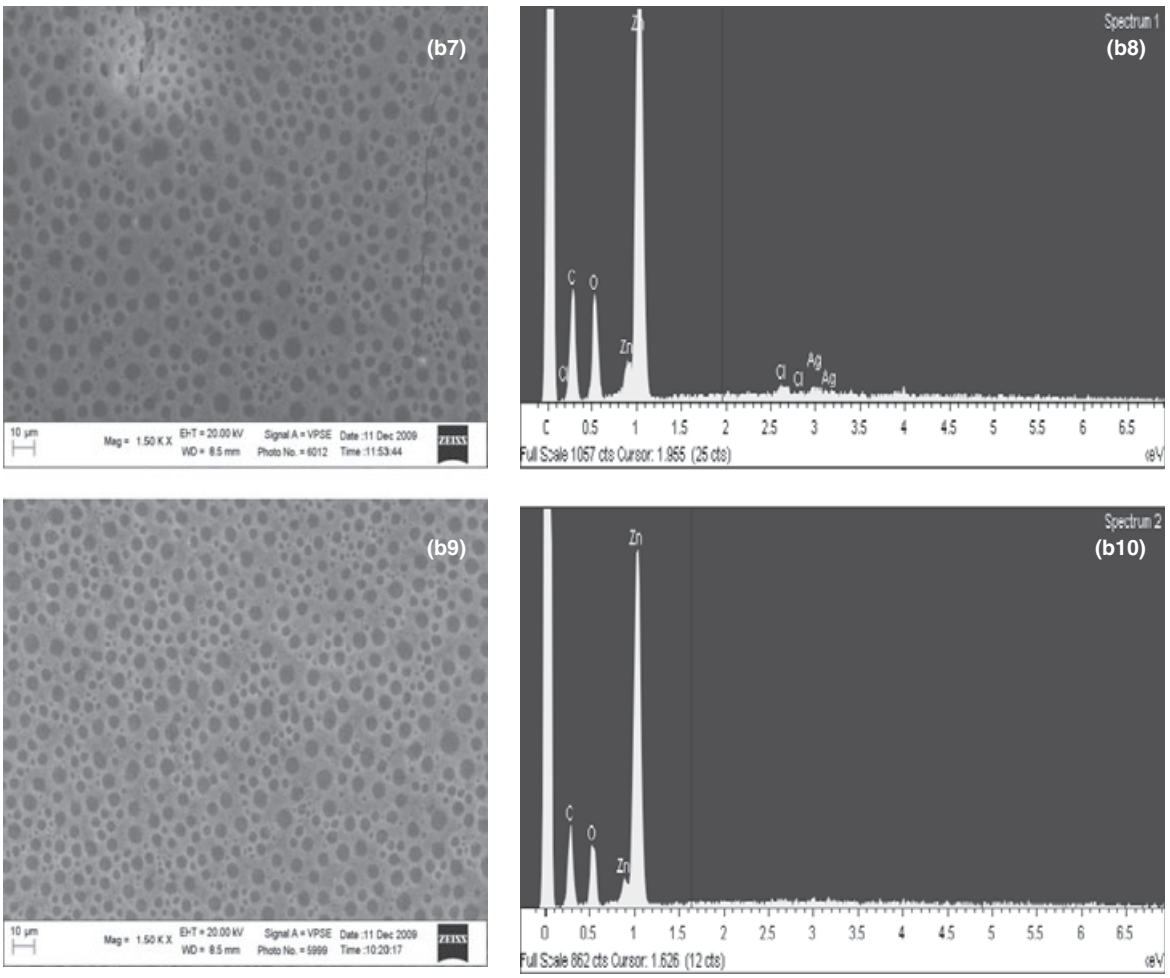

Figure 2b(7–10) (Continued).

phosphate ions were depleted over the 28-day period for MTAS but higher levels were registered for PCS. The deposition of calcium phosphates in the form of apatite and carbonated apatite has already been reported for MTA (Sarkar *et al.* 2005, Bozeman *et al.* 2006, Tay *et al.* 2007, Reyes-Carmona *et al.* 2009, Taddei *et al.* 2009, Gandolfi *et al.* 2010) and Portland cement (Coleman *et al.* 2007) in contact with simulated body fluids.

The sealing ability of the two sealers used in conjunction with gutta-percha was assessed using fluid filtration. This method is an established method used for the determination of permeability of dentine (Pashley *et al.* 1983, Tao *et al.* 1991) and has also been adapted to be used for the evaluation of sealing ability of dental materials including MTA used as a root-end filling material (Bates *et al.* 1996, Tang *et al.* 2002) and as a root canal sealer cement in conjunction with gutta-percha (Weller *et al.* 2008). It is superior to the other methods of evaluation of coronal microleakage; it is nondestructive and allows long-term evaluation of the filling. The results of sealing ability obtained using the fluid penetration method are similar to other test methods (Souza *et al.* 2008) and assessment of root fillings using bidirectional radiographs (Wu *et al.* 2009). This method does not employ the use of tracers, which may affect the sealers under test (Ahlberg *et al.* 1995, Wu *et al.* 1998b, Camilleri & Pitt Ford 2008). Fluid filtration is more reliable than the standard dye penetration method of evaluating sealing ability of root canal sealers (Camps & Pashley 2003). A pressure of 1 psi (6.895 kPa) was used in this study instead of the

28 day curing

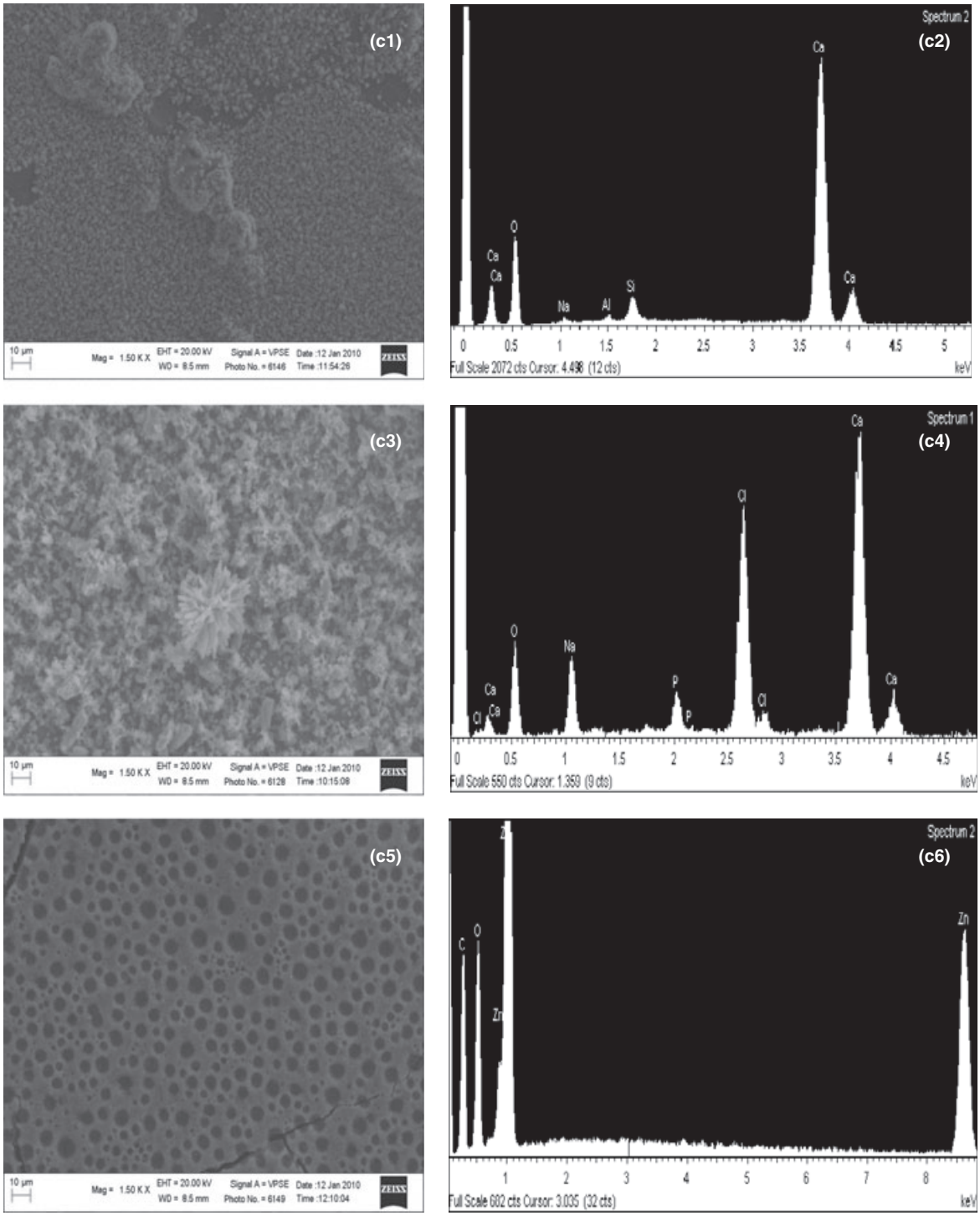

Figure 2c(1–6) (Continued).

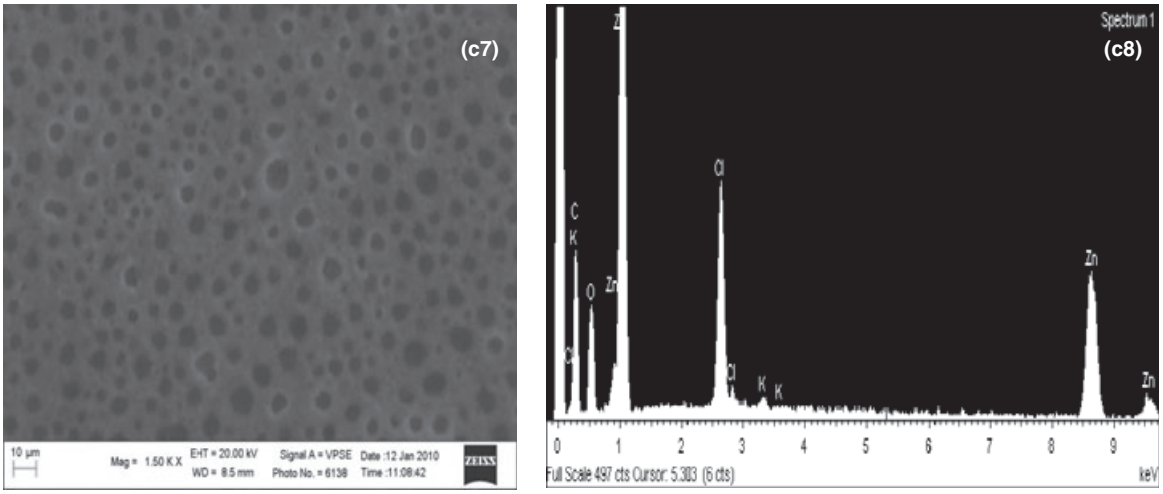

Figure 2c(7–8) (Continued).

Table 1 Elements leached out in µg g^{-1} of Pulp Canal Sealer (PCS) and mineral trioxide aggregate sealer (MTAS) after 1, 14 and 28 days in water (a), Hank's balanced salt solution (HBSS) (b)

Element detected	PCS			MTAS		
	1 day	14 days	28 days	1 day	14 days	28 days
(a)						
Ag	0.0	0.0	0.0	0.0	0.0	0.0
Al	0.0	0.0	0.0	2.1	0.2	0.0
Bi	0.0	0.0	0.0	3.7	5.6	3.9
Ca	0.0	0.0	0.0	4904	6867	7050
Si	102.9	101.0	79.3	0.0	0.0	0.0
Zn	0.8	68.6	179.4	0.0	0.0	0.0
(b)						
Ag	0.0	0.0	0.0	0.0	0.0	0.0
Al	0.0	0.0	0.0	0.5	0.0	0.0
Bi	0.0	0.0	0.0	27.2	7.4	58.9
Ca	0.0	0.0	0.0	2619	5597	5939
Si	0.0	2.2	54.1	37.2	0.0	43.4
Zn	0.0	0.0	0.0	0.0	0.0	0.0

physiological pressure through dentine, which is 1.3 kPa (Camps *et al.* 1997) to enhance apical leakage and to obtain detectable leakage values.

In this study, nickel–titanium rotary instruments were used to prepare the root canals in conjunction with gutta-percha cones that matched the taper of the canals. It has been demonstrated that the apical sealing ability of matched-taper single-cone root fillings was comparable with that of lateral condensation and Thermafil techniques (Inan *et al.* 2009). A number of publications have reported the sealing ability of PCS using the fluid filtration method (Yared & Bou Dagher 1996, Dagher *et al.* 1997, Pommel *et al.* 2003, Bouillaguet *et al.* 2008). All the different sealers tested did not fully prevent fluid flow (Bouillaguet *et al.* 2008). The sealing ability was reported to reduce with time (Bouillaguet *et al.* 2008) and also to increase with time (Dagher *et al.* 1997). PCS was also reported to have a similar or better sealing ability to resin-based sealers (Yared & Bou Dagher 1996, Pommel *et al.* 2003) and conversely in other studies using the same testing methodology it performed worse than the resin-based sealers (Adanir *et al.* 2006, Bouillaguet *et al.* 2008). In this study, the novel sealer MTAS has a sealing ability similar to PCS and the sealing ability decreased with time, whilst that of PCS showed the trend to increase with time. Conversely, research conducted on a novel

Table 2 Solution concentration of ions in parts per million (ppm) detected in Hank's balanced salt solution (HBSS) and Pulp canal Sealer (PCS) and mineral trioxide aggregate sealer (MTAS) after 1, 14 and 28 days

Element detected	HBSS	PCS			MTAS		
		1 day	14 days	28 days	1 day	14 days	28 days
Na	3289	3755.6	3725.2	3495.8	3929.3	3477.3	3303.3
P	25.4	26.1	28.7	17.1	5.1	0.0	0.37

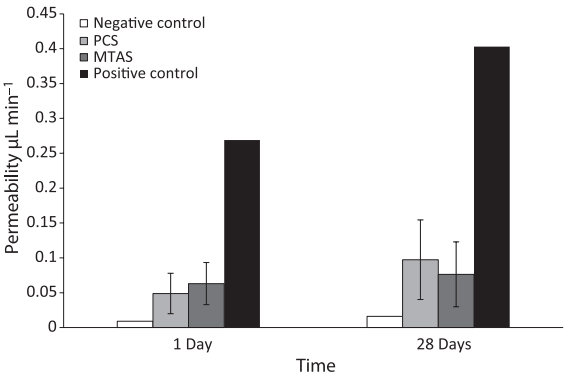

Figure 3 Permeability measurement of teeth obturated with gutta-percha and either Pulp Canal Sealer or mineral trioxide aggregate sealer immersed in HBSS after 1 and 28 days from obturation ±SD.

sealer based on MTA namely ProRoot Endo Sealer demonstrated the superior sealing ability of this material comparable to resin-based sealers and better than PCS (Weller *et al.* 2008).

Conclusions

The novel sealer based on mineral trioxide aggregate had comparable sealing ability to a proprietary brand sealer cement. In contact with a simulated body fluid, the MTAS released calcium ions in solution and encouraged the deposition of calcium phosphate crystals.

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