

Evaluation of calcium-releasing and apatite-forming abilities of fast-setting calcium silicate-based endodontic materials

L. Han, S. Kodama & T. Okiji

Division of Cariology, Operative Dentistry and Endodontics, Department of Oral Health Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Abstract

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Aim To evaluate two fast-setting calcium silicate-based endodontic materials (Endocem mineral trioxide aggregate (MTA) and Endocem Zr) with regard to their ability to release calcium ions (Ca^{2+}) and produce apatite-like precipitates after immersion in phosphate-buffered saline (PBS).

Methodology Endocem MTA, Endocem Zr and white ProRoot MTA (WMTA) were used. Chemical composition of the powder of each material was analysed with a wavelength-dispersive X-ray spectroscopy electron probe microanalyser with image observation function (SEM-EPMA). The amount of Ca^{2+} released from water-immersed set cements was measured with an EDTA titration method. Morphology and chemical composition of surface precipitates formed on the surface of PBS-immersed cements were analysed with the SEM-EPMA. Data obtained were analysed using one-way analysis of variance and Tukey's honestly

significant difference test with a significance level of 5%.

Results Endocem MTA and WMTA contained calcium (Ca), silicon (Si) and bismuth as the major elemental constituents, whereas Endocem Zr contained zirconium as the most abundant element, followed by Ca and Si. The amount of Ca^{2+} release was WMTA > Endocem MTA \geq Endocem Zr. After immersion in PBS for 14 days, the three materials produced Ca- and phosphorus (P)-containing apatite-like surface precipitates. WMTA showed higher Ca/P ratio of the precipitates compared with the other cements, with statistical significance between WMTA and Endocem Zr ($P < 0.05$).

Conclusion Compared with WMTA, Endocem MTA and Endocem Zr were associated with significantly less Ca ions release and, when immersed in PBS, produced apatite-like crystalline precipitates of significantly lower Ca/P ratios.

Keywords: apatite-forming ability, bioactivity, calcium ion release, endodontic, fast-setting calcium silicate-based cement, mineral trioxide aggregate.

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Introduction

Mineral trioxide aggregate (MTA) is a Portland cement-derived calcium silicate-based hydraulic

cement currently used for various endodontic applications such as direct pulp capping (Okiji & Yoshida 2009), root-end filling (Baek *et al.* 2010), perforation repair (Krupp *et al.* 2013) and apical barrier for teeth with open apices (Mente *et al.* 2013). A number of studies have demonstrated that MTA possesses excellent biocompatibility and sealing ability (Torabinejad & Parioikh 2010), which constitutes a basis for the wide application of this material.

A major drawback of MTA is its long setting time, which takes approximately 3 h after mixing

Correspondence: Linlin Han, Division of Cariology, Operative Dentistry and Endodontics, Department of Oral Health Science, Niigata University Graduate School of Medical and Dental Sciences, 5274 Gakkocho-dori 2-bancho, Chuo-ku, Niigata 951-8514, Japan (Tel./Fax: +81252272864; e-mail: han@dent.niigata-u.ac.jp).

(Torabinejad *et al.* 1995, Dammaschke *et al.* 2005, Gandolfi *et al.* 2009) and thus poses clinical inconvenience and puts this material at a risk of washout. Moreover, the surface structure and physical properties of MTA deteriorate when set in serum or blood (Tingey *et al.* 2008, Gandolfi *et al.* 2009, Nekoofar *et al.* 2010, Kang *et al.* 2012), which can lead to clinical failure. These drawbacks can be minimized if the cement sets quickly before it is exposed to blood or tissue fluid. Thus, studies have been conducted to shorten the setting time of MTA by the addition of setting accelerators such as calcium chloride (Kogan *et al.* 2006, Bortoluzzi *et al.* 2009), Na₂HPO₄ (Huang *et al.* 2008) and calcium lactate gluconate (Hsieh *et al.* 2009), although the acceleration does not always maintain the physical properties of MTA (Kogan *et al.* 2006, Bortoluzzi *et al.* 2009). Attempts have also been made to develop new calcium silicate-based (MTA-modified) materials with promising results (Camilleri 2008, Gandolfi *et al.* 2009, 2012, Oliveira *et al.* 2010).

Recently, two calcium silicate-based fast-setting endodontic materials have been developed. One material, Endocem MTA (Maruchi, Wonju, Korea), contains fine size particles of pozzolan, a silicate-based material that reacts with calcium hydroxide (formed after hydration of the cement) and produces additional cementitious materials. Endocem MTA has been reported to show biocompatibility similar to MTA, improved washout resistance (Choi *et al.* 2013), and cause little tooth discoloration (Jang *et al.* 2013). Another material is Endocem Zr (Maruchi, Wonju, Korea), which is a zirconium oxide-containing white MTA-like material. These materials are claimed to set within 4 min according to the manufacturer.

The performance of MTA is largely attributable to its bioactivity, that is, the capacity to release calcium ions (Ca²⁺) and produce apatite-like crystalline precipitates when in contact with phosphate-containing physiological fluids (Sarkar *et al.* 2005, Bozeman *et al.* 2006, Reyes-Carmona *et al.* 2009, Gandolfi *et al.* 2010, Han *et al.* 2010, Han & Okiji 2013). The precipitates are produced as a result of the dissolution of calcium hydroxide formed via hydration reactions; the resulting increase in pH and Ca²⁺ concentration enhances the supersaturation of phosphate-containing fluid with respect to apatite and, hence, promotes precipitation (Kokubo & Takadama 2006). The precipitates on Portland cement have been identified as calcium-deficient B-type

carbonated apatite produced via an amorphous calcium phosphate phase (Tay *et al.* 2007). The apatite formation is considered as a basis for the biocompatibility of several inorganic biomaterials such as glass ceramics (Kasuga 2005, Kokubo & Takadama 2006).

The bioactivity, that is, Ca²⁺-releasing ability and apatite-forming ability, has not yet been investigated for Endocem MTA and Endocem Zr. Thus, the aims of the present study were to analyse these materials in terms of (i) chemical composition and ultrastructure of the powder and (ii) Ca²⁺-releasing and apatite-forming abilities. The hypothesis was that the fast-setting materials do not differ from MTA with regard to the ability to release Ca²⁺ and form apatite-like surface precipitates.

Materials and methods

Materials

Endocem MTA (Lot No. B2312190225), Endocem Zr (Lot No. ZB21122601C2) and white ProRoot MTA (WMTA, Lot No. 08003394; Dentsply Tulsa, OK, USA) were used. These materials were mixed with distilled water according to the manufacturer's instruction.

Chemical composition

The powder of each material was mounted in an individual aluminium folder 4 mm in high and 6 mm in diameter and sputter-coated with a 300-Å-thick gold layer using an ion coater (IC-50; Shimadzu, Kyoto, Japan). The chemical composition and ultrastructure of the test materials were then analysed with an electron probe microanalyser (EPMA, EPMA1601; Shimadzu). All analyses were carried out at 15 kV and 100 nA of probe current, using well-characterized minerals as calibration standards. The final result for each sample was based on the average of three analyses for each material with the overall error provided by the standard deviation.

Measurement of Ca²⁺ release

Endocem MTA, Endocem Zr and WMTA were mixed with distilled water at a water-to-powder ratio of 0.12 : 0.3, 0.14 : 0.3 and 0.33 : 1.0 by weight, respectively, according to the manufacturers' instruction. A polyvinyl tube of 10 mm in length and 4 mm

in internal diameter was filled with one of the test materials ($n = 3$ in each material) and stored for 4 h at a relative humidity of 100%. The specimens were then immersed individually in 10 mL of distilled water according to the methods of Duarte *et al.* (2003) and Tanomaru-Filho *et al.* (2009). The evaluation periods were 0–5, 6–24, 25–48 and 144–168 h, and all the tubes were moved to a new solution at the beginning of each period.

The sample solutions were collected at the end of each evaluation period, and the calcium ion concentration was measured through an EDTA titration method (Itoh & Ueno 1970, Han & Okiji 2013). Briefly, 5 mL of distilled water and approximately 1 mL of a 8 molL^{-1} potassium hydroxide solution were added to 1 mL of sample solution, and then, a small amount of NN indicator ($\text{C}_{21}\text{H}_{14}\text{N}_2\text{O}_7\text{S}$; Dojindo, Kumamoto, Japan) diluted with potassium sulphate was added. The solution was titrated immediately with 0.01 molL^{-1} EDTA-2Na (Dojindo) until the wine-red colour of the solution completely disappeared and a sky-blue colour developed. The titration was repeated thrice per each sample and the mean value was calculated. The amount of calcium in the sample was calculated by the following equation; 1 mL of 0.01 molL^{-1} EDTA-2Na = 0.4008 mg Ca.

EPMA analysis of surface precipitates

Specimens prepared as mentioned above were immersed individually in plastic vials containing 20 mL of Ca- and Mg-free phosphate-buffered saline (PBS; 136.4 mmolL^{-1} NaCl, 2.7 mmolL^{-1} KCl, 8.2 mmolL^{-1} NaH_2PO_4 and 1.25 mmolL^{-1} KH_2PO_4 in 1000 mL of distilled water; pH 7.4) for 14 days. The PBS was replaced every 3 days. Surface precipitates formed on the specimens were harvested by gentle

scraping with a sterile dental mixing spatula. Morphology and elemental composition of the collected precipitates were mounted on aluminium stubs, sputter-coated as described above and analysed using the EPMA at an accelerating voltage of 15 KV, as described previously (Han & Okiji 2013).

Statistics

Data obtained were analysed using one-way analysis of variance (ANOVA) and Tukey's honestly significant difference test with a significance level of 5%.

Results

Elemental composition and morphology of cement powder

The analytic data obtained through EPMA are shown in Fig. 1 and Table 1. WMTA and Endocem MTA contained three specific elements, that is, Ca, Si and Bi (Table 1). Particles of calcium silicate and bismuth oxide were found in both materials, whilst particles representing pozzolans were detected only in Endocem MTA (Fig 1). On the other hand, Endocem Zr contained Ca, Si and, notably, a high content of Zr (57.49 wt%) instead of Bi (Table 1). Morphologically, fine particles of zirconium oxide were detected (Fig 1). In addition, Endocem MTA and Endocem Zr contained higher percentages of aluminium (Al) and iron (Fe) compared with WMTA (Table 1).

Ca^{2+} release

Table 2 shows the concentration of calcium ion released from each material at each time period. At all the time periods, WMTA showed a significantly

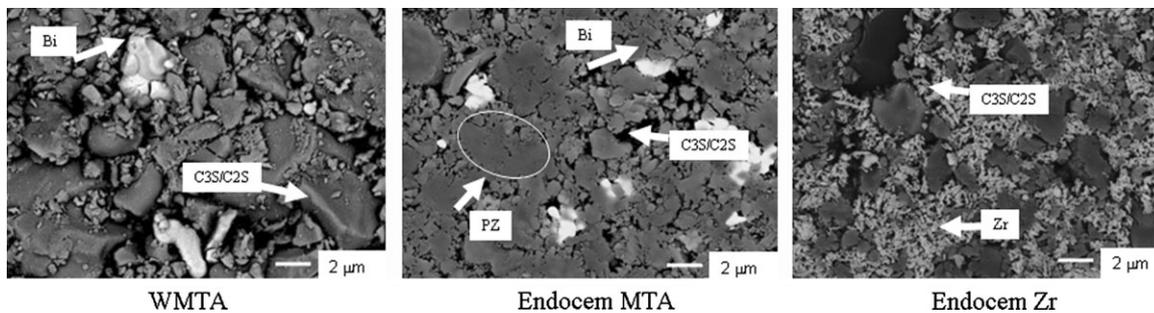


Figure 1 Ultrastructure of WMTA, Endocem MTA and Endocem Zr. C3S/C2S: calcium silicate; Bi: bismuth oxide; PZ: pozzolan; Zr: zirconium oxide.

Table 1 Major elemental composition of WMTA, Endocem MTA and Endocem Zr (wt% conc.)

Elements	WMTA		Endocem MTA		Endocem Zr	
	Mean	SD	Mean	SD	Mean	SD
Ca	37.18	1.97	40.21	3.12	18.52	0.91
O	32.98	3.15	15.34	1.12	11.7	0.78
Si	11.17	0.96	10.2	2.15	4.82	0.25
Bi	15.75	0.12	24.24	0.12	ND	–
Zr	ND	–	ND	–	57.49	3.11
S	1.11	0.02	1.99	0.16	0.86	0.07
Al	0.93	0.05	3.33	0.21	1.45	0.06
Fe	0.54	0.05	2.92	0.3	1.63	0.05
Mg	0.36	0.01	ND	–	0.81	0.02

N = 3; ND: Not detected.

higher Ca²⁺ concentration as compared with the other materials (*P* < 0.05). Endocem MTA showed values significantly higher than Endocem Zr at 6–24 h and 144–168 h (*P* < 0.05).

Morphology and element composition of precipitates

The three materials produced surface crystalline precipitates typically showing a spherical appearance with acicular microprojections (Fig. 2). The precipitates on the three materials contained Ca and P as the main constituents, and the Ca/P ratio of the precipitate was in the order of WMTA >Endocem MTA >Endocem Zr, with statistical significance between Endocem Zr and the other materials (*P* < 0.05; Table 3).

Discussion

This study demonstrated that all the three materials tested released Ca²⁺ and formed Ca- and P-rich surface precipitates. Compared with WMTA, however, the two fast-setting materials were associated with significantly less Ca²⁺ release and produced precipitates with significantly lower Ca/P ratios. Thus, the

hypothesis that the fast-setting materials do not differ from WMTA with regard to the ability to release Ca²⁺ and form apatite-like surface precipitates was rejected.

In this study, all the test materials released Ca²⁺. This was determined by EDTA titration, which is a practical method applied in various fields of analytic chemistry (Itoh & Ueno 1970). The results demonstrated that Ca²⁺ release from WMTA was initially high and decreased gradually over time, which is consistent with other studies where atomic absorption spectrophotometry (Duarte *et al.* 2003, Camilleri 2008) and EDTA titration (Han & Okiji 2013) were employed. The significantly lower levels of Ca²⁺ release from the two fast-setting materials may be attributed to the composition and setting reaction of these materials that are different from those of WMTA, as will be discussed below. Another possibility is that the solubility of the fast-setting materials is lower than that of WMTA, which is suggested by a report showing that Endocem MTA has a higher washout resistance than WMTA (Choi *et al.* 2013), but not yet determined for Endocem Zr.

Endocem MTA employs the pozzolanic reaction, which is the chemical reaction of pozzolan with calcium hydroxide to form compounds possessing cementitious properties and known to reduce the setting time of Portland cements (Snellings *et al.* 2012). During the pozzolanic reaction, Portland cements have a gradual decrease in the amount of free calcium hydroxide and increase in the formation of stable crystals of calcium silicate hydrate and calcium aluminate hydrate, both of which may confer mechanical strength of the material (Rodríguez-Camacho & Uribe-Afif 2002, Turanli *et al.* 2004). The present analysis detected fine pozzolan-like particles in the powder of Endocem MTA (Fig. 1) and further demonstrated that this material contains a higher concentration of aluminium (Table 1), part of which may be derived from pozzolan and involved in the reduction in setting time (Rodríguez-Camacho & Uribe-Afif 2002, Turanli *et al.* 2004). The use of fine pozzolan

Table 2 Concentration of Ca²⁺ released from set materials (mg L⁻¹)

Materials	0–5 h		6–24 h		25–48 h		144–168 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
WMTA	18.61 ^a	0.78	24.90 ^a	1.63	20.24 ^a	1.76	9.37 ^a	1.27
Endocem MTA	6.95 ^b	0.85	11.27 ^b	1.11	7.30 ^b	0.73	6.95 ^b	0.84
Endocem Zr	5.79 ^b	0.6	9.67 ^c	0.67	5.51 ^b	1.16	4.94 ^c	0.72

N = 3; Different superscripts in each column are significantly different (*P* < 0.05).

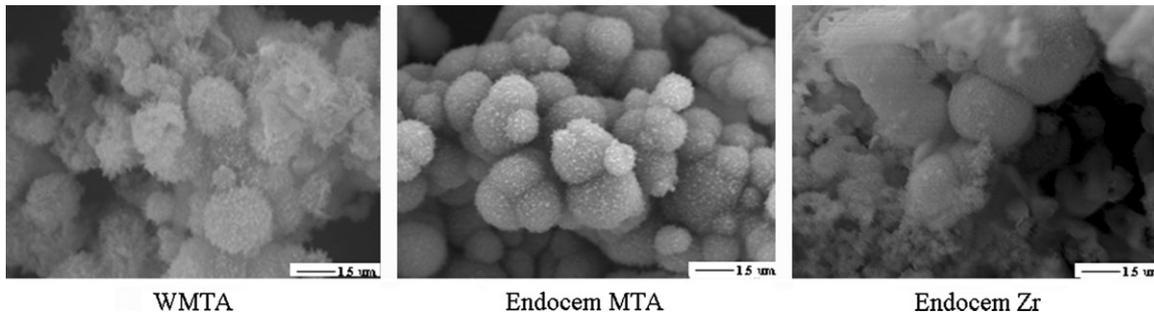


Figure 2 Ultrastructure of surface precipitates on set WMTA, Endocem MTA and Endocem Zr produced after immersion in PBS for 14 days.

particles may increase the surface contact with water and may provide rapid setting and ease of handling (Mostafa *et al.* 2010, Askarinejad *et al.* 2012). In the present analysis, however, Endocem MTA released a significantly smaller amount of Ca^{2+} compared with WMTA at all time periods examined (Table 2). This may be attributed, at least in part, to the consumption of calcium hydroxide during the pozzolanic reaction, because the progress of the pozzolanic reaction reduces the amount of free calcium hydroxide produced during hydration (Snellings *et al.* 2012).

Endocem Zr is a zirconium oxide-enriched calcium silicate-based cement and its high Zr content (57.49 wt%) was indeed demonstrated (Table 1). Zirconium oxide has been employed as a radiopacifier of calcium silicate-based endodontic materials (Camilleri *et al.* 2011, Viapiana *et al.* 2014) as a substitute of bismuth oxide, because bismuth oxide retards the setting reaction of MTA (Coomaraswamy *et al.* 2007). Zirconium oxide nanoparticles have also been reported to accelerate the degree of hydration of Portland cements (Nazari & Riahi 2010, Li *et al.* 2013). Thus,

the replacement of bismuth oxide with zirconium oxide may contribute to the fast-setting property of Endocem Zr. However, the addition of a high amount of zirconium oxide to Endocem Zr greatly reduced the relative concentration of Ca and Si in this material (Table 1). Such a low Ca concentration may be the primary reason of the present finding that Endocem Zr had significantly lower Ca^{2+} release compared with the other materials tested (Table 2).

In the present study, the ultrastructural examination of PBS-immersed specimens of all test materials allowed verification of the presence of precipitates of predominantly acicular morphology in the presence of PBS (Fig. 2). The chemical composition of the precipitates was similar between the three materials, in that Ca and P were the main components (Table 3). These findings are consistent with a number of previous studies where precipitate formation on MTA has been examined (Sarkar *et al.* 2005, Bozeman *et al.* 2006, Reyes-Carmona *et al.* 2009, Gandolfi *et al.* 2010, Han *et al.* 2010, Han & Okiji 2013). These findings also indicate that the two fast-setting materials and WMTA share the ability to form apatite-like precipitates in the phosphate-containing environment, which is considered as a basis for the biocompatibility of these materials (Kokubo & Takadama 2006). Studies have also disclosed that the precipitates are formed along the MTA-dentine interface and within the interfacial dentine (Sarkar *et al.* 2005, Reyes-Carmona *et al.* 2009, Han & Okiji 2011, 2013), which leads to the notion that they contribute to reducing leakage not only by filling the gap along the interface but also via interactions with dentine such as intrafibrillar apatite deposition.

However, the two fast-setting materials produced precipitates with lower Ca/P ratio compared with WMTA. This may be attributable to the lower

Table 3 Major chemical compositions and Ca/P ratio of the surface precipitates on PBS-immersed test materials

Elements	WMTA		Endocem MTA		Endocem Zr	
	wt% conc	SD	wt% conc	SD	wt% conc	SD
Ca	39.85	1.79	37.71	2.55	27.44	1.16
P	22.81	1.48	24.33	2.27	20.19	1.55
Si	3.22	0.17	0.43	0.05	0	0
O	22.01	1.77	34.02	5.62	28.93	2.02
Ca/P	1.75 ^a	0.12	1.55 ^{ab}	0.11	1.36 ^b	0.12

N = 3; The same superscripts in Ca/P are not significantly different (*P* > 0.05).

Ca-releasing ability of these materials compared with WMTA. This notion is supported by the fact the growth and nucleation of the apatite layer are proportional to the concentration of available ions (Weng et al. 1997).

Overall, the results of the present study suggest that Endocem MTA and Endocem Zr share the Ca-releasing and apatite-like precipitate-forming abilities with WMTA. Whether the lower Ca-releasing ability and resulting lower Ca/P ratio of the precipitates has an influence on the biological and clinical performance of the two fast-setting materials requires further evaluation.

Conclusions

Compared with WMTA, Endocem MTA and Endocem Zr were associated with less Ca ion release and produced apatite-like crystalline precipitates of significantly smaller Ca/P ratios when immersed in PBS.

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