Characterization of set Intermediate Restorative Material, Biodentine, Bioaggregate and a prototype calcium silicate cement for use as root-end filling materials

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Abstract

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Aim To investigate the composition of materials and leachate of a hydrated prototype cement composed of tricalcium silicate and radiopacifier and compare this to other tricalcium silicate-based cements (Biodentine and Bioaggregate) to assess whether the additives in the proprietary brand cements affect the hydration of the materials, using Intermediate Restorative Material (IRM), a standard root-end filling material as a control.

Methodology The materials investigated included a prototype-radiopacified tricalcium silicate cement, Biodentine, Bioaggregate and Intermediate Restorative Material (IRM). The pH and calcium ion concentration of the leachate were investigated. The hydrated cements were characterized using scanning electron microscopy (SEM) and X-ray energy dispersive analysis (EDX), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR).

Results All the cements tested were alkaline. The tricalcium silicate-based cements leached calcium in solution. Scanning electron microscopy of the prototype-radiopacified tricalcium silicate cement, Biodentine and Bioaggregate displayed hydrating cement grains, surrounded by a matrix composed of calcium silicate hydrate and calcium hydroxide. The presence of calcium hydroxide was evident from the XRD plots. FT-IR indicated the occurrence of a poorly crystalline calcium silicate hydrate. Biodentine displayed the presence of calcium carbonate. Bioaggregate incorporated a phosphate-containing phase. IRM consisted of zinc oxide interspersed in an organic matrix.

Conclusions The hydration of prototype-radiopacified tricalcium silicate cement, Biodentine and Bioaggregate resulted in the formation of calcium silicate hydrate and calcium hydroxide, which was leached in solution. The hydrated materials were composed of a cementitious phase that was rich in calcium and silicon and a radiopacifying material. Biodentine included calcium carbonate, and Bioaggregate included silica and calcium phosphate in the powders. IRM was composed of zinc oxide interspersed in a matrix of organic material.

Keywords: Bioaggregate, Biodentine, characterization, hydration, leaching, tricalcium silicate cement.

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Introduction

A number of materials have been developed by various manufacturers for use as a root-end filling materials. The first material to be developed specifically for this purpose was mineral trioxide aggregate (MTA) whilst more recently materials based on tricalcium

silicate cement have been introduced. Such materials include Biodentine (Septodont, Saint-Maur-des-fossés, France) and Bioaggregate (Innovative Bioceramix Inc. Vancouver, Canada; http://www.ibioceramix.com/Bio-Aggregate.html). This class of materials is characterized by the release of calcium hydroxide in solution (Camilleri 2008, 2011), which when in contact with tissue fluids forms hydroxyapatite (Tay *et al.* 2007, Taddei *et al.* 2009, Han *et al.* 2010).

Clinically, the composition of the set material is important. Once placed and in contact with tissue fluids, root-end filling materials can leach trace elements and compounds that can be affect the surrounding periapical tissues. The literature available on Biodentine and Bioaggregate is limited with the reports on the material composition restricted to the manufacturer's data sheets.

Both Biodentine and Bioaggregate are primarily composed of tricalcium silicate cement and a radiopacifier phase, which is zirconium oxide in Biodentine and tantalum oxide in Bioaggregate. The tricalcium silicate cement is guaranteed for purity as it is manufactured in a laboratory using the sol-gel method (Sorrentino 2008, Chen *et al.* 2009). Biodentine powder also contains calcium carbonate (filler material) whilst the liquid supplied for mixing with the cement powder consists of calcium chloride (decreases the setting time) and a hydrosoluble polymer (water-reducing agent) (Biodentine Scientific File 2010). Analysis of the powder and liquid using X-ray fluorescence spectroscopy (XRF) displayed the presence of calcium, silicon and zirconium in the powder and sodium, magnesium, chloride and calcium together with the water in the liquid phase (Camilleri *et al.* 2012). This analysis verifies the presence of the main constituent elements, but provides no indication of the phases present in the material.

Bioaggregate also includes hydroxyapatite and amorphous silicon oxide together with the main cementitious and radiopacifier phases (Innovative Bioceramix Inc.). The hydroxyapatite and amorphous silicon oxide are added to reduce the levels of the calcium hydroxide produced on hydration, which is a weak phase. Calcium hydroxide is leached in solution and is not cementitious. Thus, it does not contribute to the strength of the material. The crystal structure has been verified by X-ray diffraction analysis (Park *et al.* 2010) where all the crystalline phases were identified and X-ray fluorescence spectroscopy (Camilleri *et al.* 2012), which displayed the presence of the different elements present in oxide form namely tantalum, silicon, calcium and phosphorus. The absence of aluminium

specified by the manufacturer was also verified (Park *et al.* 2010, Camilleri *et al.* 2012). The liquid provided with Bioaggregate was composed of pure water (Camilleri *et al.* 2012). Hydration of Bioaggregate resulted in the formation of calcium hydroxide. Calcite was also observed in the set forms of the material but not in the unhydrated powder (Park *et al.* 2010).

The lack of heavy element contamination in tricalcium silicate cement and also in Biodentine and Bioaggregate has been verified by inductively coupled plasma analysis of acid extracts and leached species (Camilleri *et al.* 2012). Tricalcium silicate cement was mostly used as a bone cement (Huan & Chang 2007, 2008) and more recently has been introduced for use as a dental material (Wang *et al.* 2008, Chen *et al.* 2009). Tricalcium silicate cement reacts with water to produce calcium-silicate-hydrate gel and calcium hydroxide (Camilleri 2011). The leaching of calcium is enhanced when the cement is in contact with physiological solutions and forms a layer of hydroxyapatite on the cement surface (Camilleri 2011). The cement grain reacts with water and forms a reaction rim with calcium hydroxide being deposited in the cement matrix. The pores of the calcium silicate hydrate provide nucleation sites for calcium hydroxide crystals, from where these crystals grow. As the crystals age, the calcium silicate hydrate polymerizes and hardens (Li & Roy 1988).

Intermediate Restorative Material (IRM) is reinforced zinc oxide eugenol cement that has various applications in dentistry. One of its uses is as a root-end filling material. IRM has shown a success rate comparable to mineral trioxide aggregate when used as a root-end filling material (Chong *et al.* 2003). Despite its extensive use, there is very little literature on this material.

The aim of this research was to investigate the composition and leachate of a hydrated prototype cement composed of tricalcium silicate and radiopacifier and compare this to other tricalcium silicate-based cements (Biodentine and Bioaggregate) to assess whether the additives in the proprietary brand cements affect the hydration of the materials, using IRM, a standard root-end filling material as a control.

Materials and methods

The materials used in this study included:

Tricalcium silicate cement (Mineral Research Processing, Meyzieu, France) replaced with 20% zirconium oxide by weight (ZrO_2; Sigma-Aldrich, Buchs, Germany) – TCS-20-Z;

Biodentine (Septodont, Saint-Maur-des-fossés Cedex, France);

Bioaggregate (Innovative Bioceramix Inc. Vancouver, Canada);

Intermediate Restorative Material (Dentsply DeTrey, Konstanz, Germany) - IRM;

The TCS-20-Z was mixed at a water to cement ratio of 0.35. The water/cement ratio as set at 0.35 as this resulted in the best consistency (Camilleri 2011). The water/powder ratio was 0.28 because the zirconium oxide is noncementitious (Cutajar *et al.* 2011, Formosa *et al.* 2012b). The Biodentine, Bioaggregate and IRM were mixed according to manufacturer's instructions. The materials were soaked in Hank's balanced salt solution (HBSS; H6648, Sigma-Aldrich, St. Louis, MO, USA) for 28 days at 37 °C in an incubator. The composition of the HBSS comprised (g/L) 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.

Evaluation of pH and calcium ion concentration in solution

Discs of diameter 15 ± 1 mm and a thickness of 1 ± 0.1 mm of each cement type were prepared. The cements were immediately immersed in 10 mL HBSS and pH and calcium ion readings of the storage solution were taken using a pH/mV/ISE meter (Hanna HI 3221; Hanna Instruments, Woonsocket, RI, USA) with a single-junction (Ag/AgCl) ceramic pH electrode (Hanna HI 1131; Hanna Instruments) and ISE Calcium Electrode (consisting of Hanna HI 4000-50 Sensor handle and Hanna HI 4004-51 calcium module) and a reference electrode (Hanna HI 5315; Hanna Instruments), respectively. The pH and the calcium ion concentration of each solution were measured after 1, 7, 14, 21 and 28 days postimmersion. Three replicate samples of each of the four materials were prepared for two tests ($n = 24$).

X-ray diffraction analysis

Phase analysis was carried out on the cements using X-ray diffraction (XRD). The diffractometer (Rigaku, Tokyo, Japan) used Cu Kα radiation at 40 mA and 45 kV. The cement pastes were crushed using a mortar and pestle prior to testing. Samples were presented in powder form, and the detector was set to rotate between 5 and 45°, a sampling width of 0.05° and scan speed of 2° per minute. Phase identification was accomplished using a search-match software utilizing ICD database (International Centre for Diffraction Data, Newtown Square, PA, USA).

Fourier transform infrared (FT-IR) spectroscopy

The hydrated cements immersed in HBSS for 28 days were desiccated and crushed to powder with a pestle and mortar. Two to five mg of cement was added to 100 mg potassium bromide and analysed using a Fourier transform infrared spectrophotometer (Shimadzu IRAffinity-1; Shimadzu Corp., Kyoto, Japan) using transmitted infrared spectroscopy.

Microscopy and elemental analysis

Cubes $7 \times 7 \times 7$ mm were prepared and immersed immediately in HBSS for 28 days at 37 ± 1°C after which they were taken out of solution, dried using a filter paper and were placed in a vacuum desiccator containing silica crystals and soda lime for 8 h. The specimens were then mounted in resin (Epoxyfix, Struers GmbH, Ballerup, Denmark) and then were ground using progressively finer diamond discs and pastes using an automatic polishing machine (Roto-Pol-31; Struers GmbH) prior to mounting on aluminium stubs, carbon coated and viewing under the scanning electron microscope (SEM; Zeiss MERLIN Field Emission SEM, Carl Zeiss NTS GmbH, Oberkochen, Germany). Scanning electron micrographs of the different material microstructural components at different magnifications in back-scatter electron mode were captured, and X-ray energy dispersive analysis (EDX) of the different phases was carried out.

Statistical analysis

The data were evaluated using SPSS (Statistical Package for the Social Sciences) software (PASW Statistics 18; SPSS Inc., Chicago, IL, USA). Parametric tests were performed as K-S tests on the results indicated that the data were normally distributed. Analysis of variance (ANOVA) with $P = 0.05$ and Tukey's *post hoc* test were used to perform multiple comparison tests.

Results

Evaluation of pH and calcium ion concentration in solution

The results for the pH values of the leachate from cement samples immersed in HBSS for 28 days are
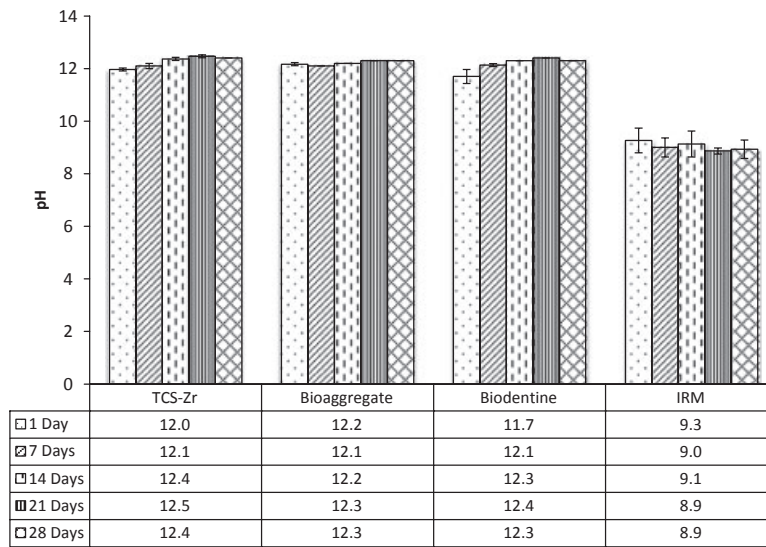
Figure 1 pH of leachate after immersion in Hank's balanced salt solution for 28 days.

shown in Fig. 1. There was no difference in the pH values for each material over the 28-day period ($P > 0.05$). The pH of the solution in contact with IRM was approximately 9, significantly lower than the pH of the tricalcium silicate-based cements, which was 12 at all-time intervals ($P < 0.001$).

The results for the determination of leaching of calcium ions in solution are shown in Fig. 2. All the tricalcium silicate-based materials leached calcium ions in solution. The leaching of calcium increased from Day 1 reaching a plateau by Day 14 where the calcium levels were maintained until Day 28. IRM did not leach calcium. Calcium ion release by Biodentine was higher than all the other material tested ($P < 0.001$). Bioaggregate had the lowest level of calcium ions in solution compared to the other tricalcium silicate-based cements ($P < 0.001$).

Characterization and phase analysis of the hydrated cements

The scanning electron micrographs and EDX analysis are shown in Fig. 3 whilst the X-ray diffractograms and FT-IR plots for all the test materials are shown in Fig. 4. All the materials were composed of two basic phases. The tricalcium silicate-based cements (Fig. 3a–c1) were composed of a cement phase, which exhibited elemental peaks for calcium, silicon and oxygen (Fig. 3a–c3) and a radiopacifier phase, which displayed peaks for zirconium in the radiopacified trical-

cium silicate cement (Fig. 3a2) and Biodentine (Fig. 3c2) and tantalum in Bioaggregate (Fig. 3b2). The cement matrix of Bioaggregate (Fig. 3b3) also displayed elemental peaks for phosphorus.

The radiopacified tricalcium silicate cement exhibited larger unreacted cement particles when compared to Biodentine. These cement particles exhibited reaction rims at their circumference. The zirconium oxide particles were small and had a tendency to agglomerate (Fig. 3a1). In contrast, Biodentine exhibited minute cement particles, which could not be discerned from the hydration product. The calcium carbonate particles were large (around 2–3 microns) in comparison with the cement particles. Although the calcium carbonate was noncementitious, it exhibited reaction rims of hydration product around its circumference. There was less zirconium oxide in Biodentine when compared to the radiopacified tricalcium silicate cement. Bioaggregate exhibited hydrating cement particles with associated reaction by-products around the cement particle and in the cement matrix (Fig. 3a2). Tantalum oxide demonstrated a large particle size (Fig. 3a2 inset). IRM was composed of fine shiny particles, which were in turn mixed with darker particles (Fig. 3d1). The shiny particles exhibited a zinc peak on EDX analysis (Fig. 3d2). There were also large areas rich in carbon when tested using EDX analysis (Fig. 3d3).

The X-ray diffraction plots presented the tricalcium silicate phase (ICDD: 31-0301) for all the tricalcium
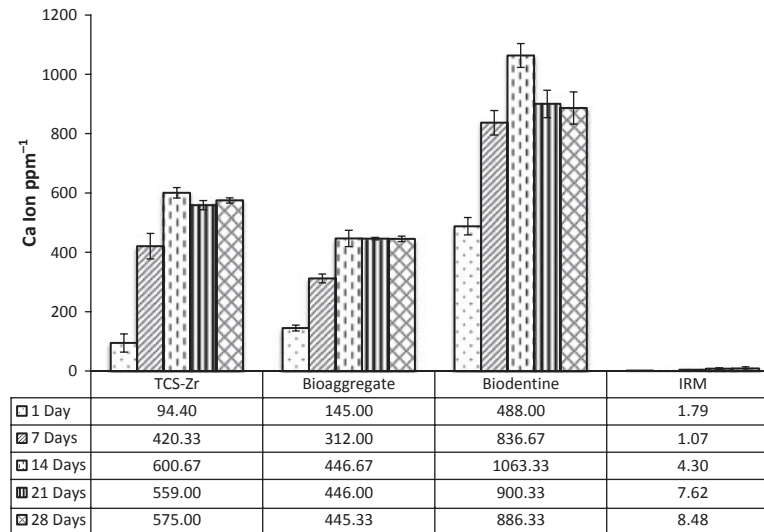
	TCS-Zr	Bioaggregate	Biodentine	IRM
□ 1 Day	94.40	145.00	488.00	1.79
▨ 7 Days	420.33	312.00	836.67	1.07
▥ 14 Days	600.67	446.67	1063.33	4.30
▤ 21 Days	559.00	446.00	900.33	7.62
▨ 28 Days	575.00	445.33	886.33	8.48

Figure 2 Calcium ion release in solution of test materials after immersion in Hank's balanced salt solution for 28 days.

silicate cement-based materials (Fig. 4) with peaks at 29.35, 32.461, 34.27° 2θ. The peaks were not very well defined compared to the radiopacifier peaks. All the tricalcium silicate-based cements displayed a wavy baseline signifying the presence of an amorphous phase. In addition, the cements based on tricalcium silicate exhibited a portlandite (ICDD: 44-1481) peak at 18.05 and 34.09° 2θ. The portlandite (calcium hydroxide) peak for the radiopacified tricalcium silicate cement at 18.05° 2θ was more intense than for Bioaggregate and Biodentine. Biodentine and TCS-20-Z cement exhibited peaks for the zirconium oxide phase (ICDD: 37-1484) at 28.17 and 31.47° 2θ. The zirconium oxide peak in Biodentine was less intense than that of TCS-20-Z. Biodentine also exhibited a peak for calcium carbonate (ICDD: 05-0586) at 29.35° 2θ. Tantalum oxide (ICDD: 33-1390) was present at 23.50, 26.33, 29.81 and 36.33° 2θ in Bio-aggregate.

The FT-IR spectra the calcium silicate-based cements namely the radiopacified tricalcium silicate, Bioaggregate and Biodentine (Fig. 4) exhibited a characteristic set of bands at the range 973–981 cm^{-1}, which are attributed to tricalcium silicate hydrate. These are the most intensive bands in all spectra and can be assigned to Si-O stretching. In addition, very weak bands in the range 821–842 cm^{-1} assigned also to Si-O stretching vibrations were also present. The splitting of the band in the 1000–850 cm^{-1} region results from the presence of silicate phases.

The bands in the region of 1640 cm^{-1} are due to H-O-H bending vibrations of H_2O molecules. Calcium hydroxide, which is a by-product of hydration of tricalcium silicate cement, also exhibited bands in the region of 1600–1400 cm^{-1}. The bands in the 1600–1300 cm^{-1} correspond to the asymmetric stretching of CO_3^{2-}. Carbonate phases were present in Biodentine as verified by the SEM and XRD analysis. Calcium carbonate could also be formed on the other cements from the surface carbonation of cements by reaction of the calcium hydroxide with the atmospheric carbon dioxide.

Phase analysis of IRM with XRD indicated crystalline phases (ICDD: 03-0888) with definite peaks at 31.82, 34.47, 36.19, 47.57° 2θ. The FT-IR spectrum of IRM (Fig. 4h) indicated the details of functional groups present in the organic phase. A sharp intense peak at 1731 cm^{-1} appeared due to the presence of ester carbonyl group stretching vibration typical of polymethyl methacrylate. The broad band from 950 to 650 cm^{-1} is due to the bending of C-H. The FT-IR spectrum also showed the presence of alcohol group at 3462 cm^{-1} and methoxy group at 1149 cm^{-1} typical of eugenol.

Discussion

The materials investigated in this study included two commercially available tricalcium silicate-based cements and a prototype cement. Tricalcium silicate cement displays comparable physical and chemical properties to mineral trioxide aggregate (Camilleri 2011, Formosa *et al.* 2012a,b). In addition, zirconium oxide has been used as an alternative radiopacifier
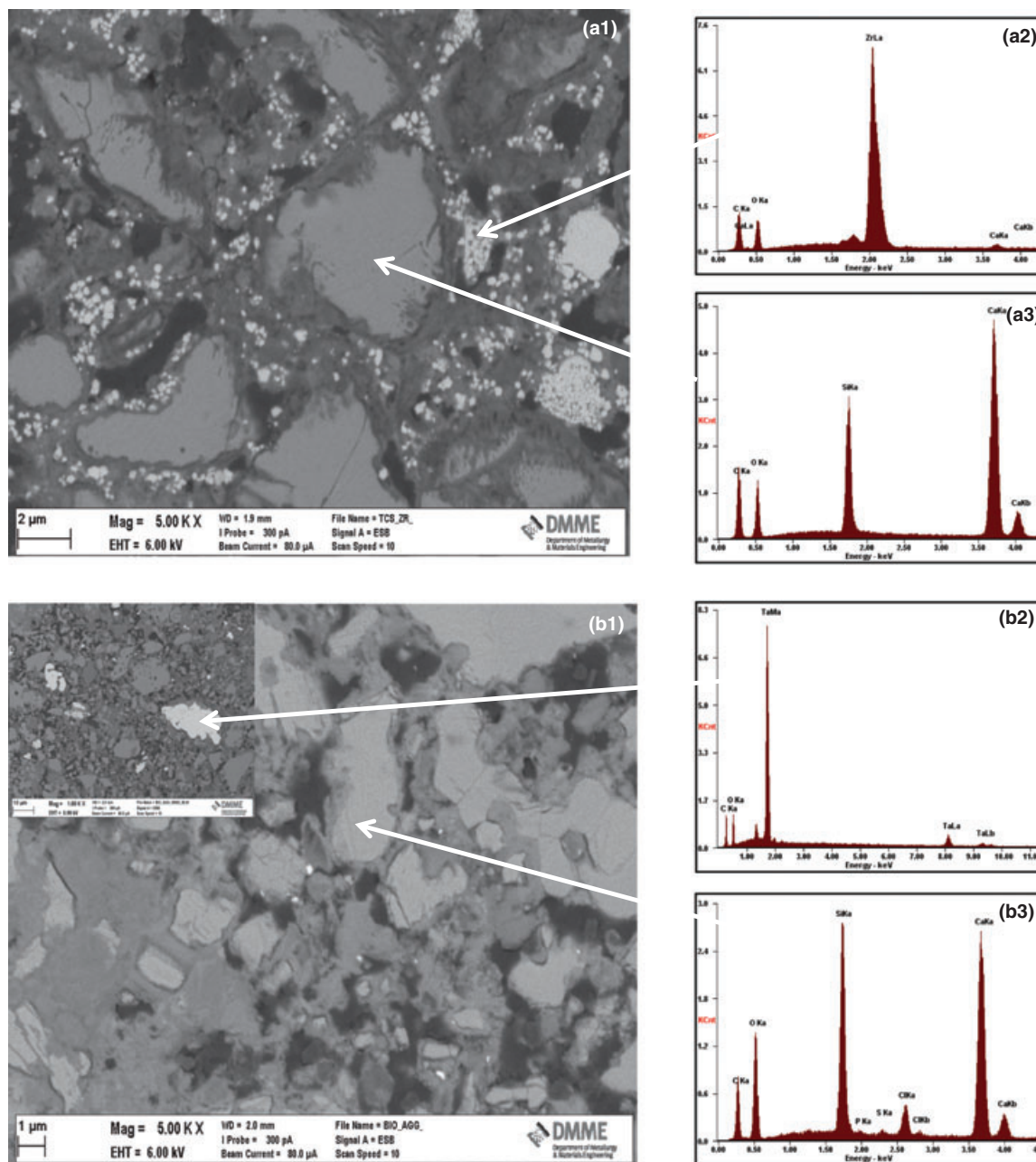
Figure 3 Characterization of hydrated cements (a) tricalcium silicate and 20% zirconium oxide; (b) Bioaggregate; (c) Biodentine; (d) Intermediate Restorative Material (IRM) showing (1) scanning electron micrographs; (2, 3) elemental investigation using EDX.

with calcium silicate-based cements resulting in optimal properties (Camilleri *et al.* 2011, Cutajar *et al.* 2011). The prototype cement was a mixture of tricalcium silicate cement and 20% zirconium oxide. Thus, it was similar to Biodentine; however, it did not incorporate the additives in the powder and liquid. This prototype cement was created to assess the effects that the additive components had on Biodentine. IRM, which is used routinely as a root-end filling material and has shown a high success rate comparable to mineral trioxide aggregate (Chong *et al.* 2003), was used as the control.
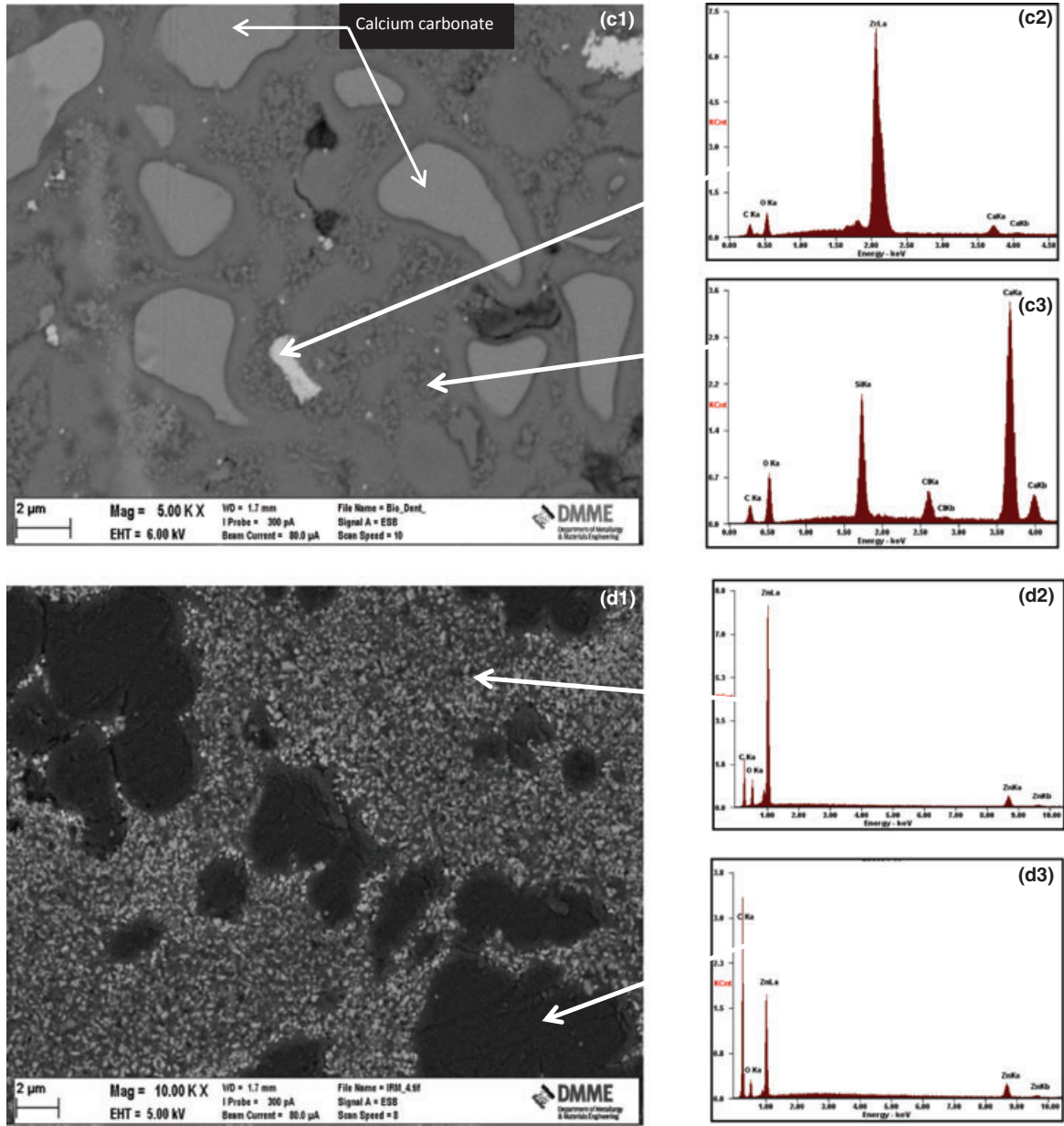
Figure 3 Continued.

The setting characteristics of the test materials were assessed after storage in simulated body fluid for 28 days. The set prototype cement, Bioaggregate and Biodentine were composed of two phases namely the cementitious component, which was verified to be made up of tricalcium silicate from the EDX and XRD analysis. The EDX identified the different elements whilst the specific phases were confirmed by XRD, which gives specific peaks that are particular to the different phases present in each test material. A radiopacifier phase was present in each cement. Biodentine contained zirconium oxide whilst Bioaggregate included tantalum oxide. Both these phases were verified by XRD analysis. The amount of zirconium oxide present in the Biodentine was less than in the prototype cement that comprised 20% zirconium oxide. Portland cement with various additions of zirconium oxide has been assessed and 20% zirconium oxide
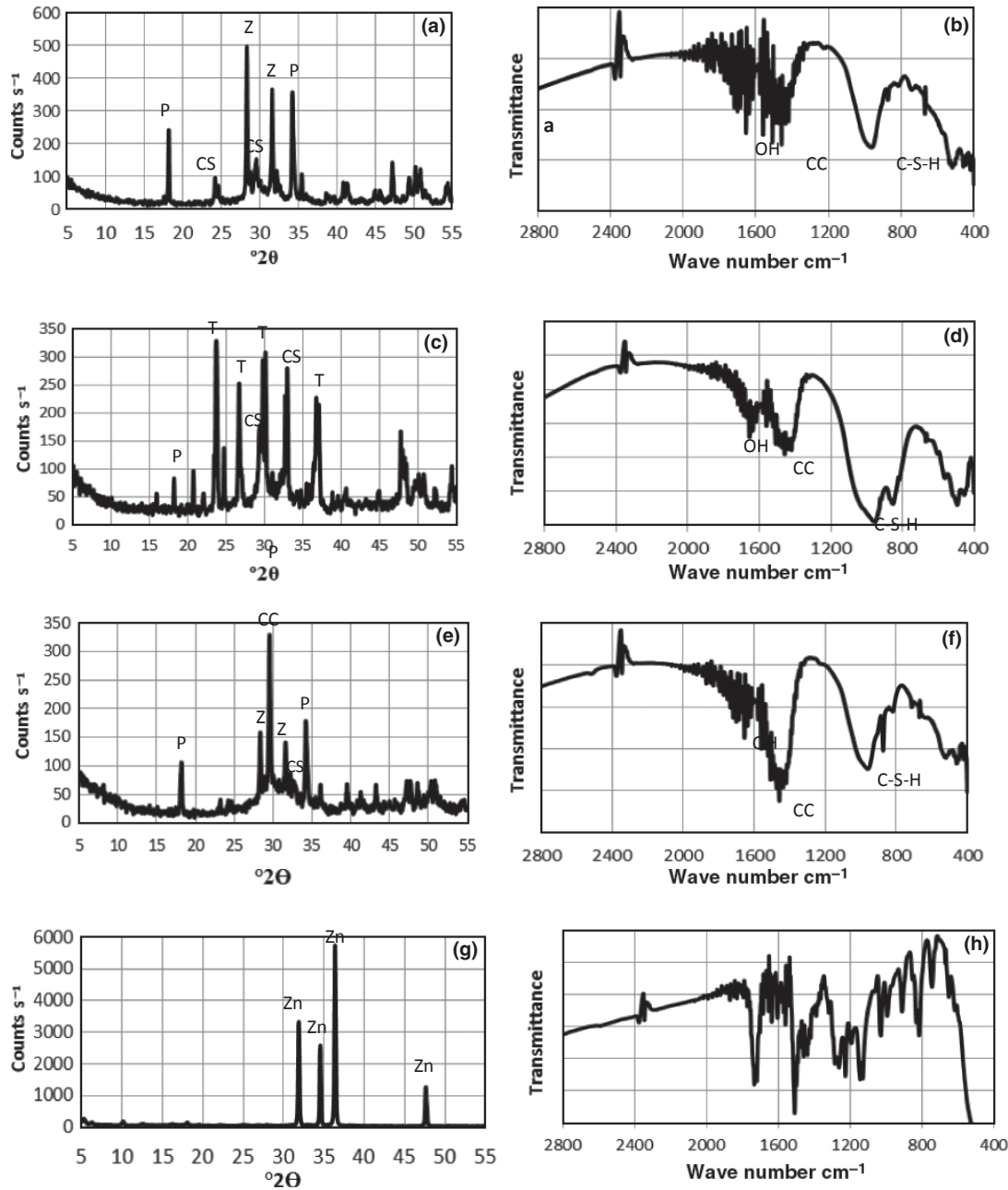
Figure 4 (a, c, d, f) Phase analysis with X-ray diffraction and (b, d, f, h) Fourier transform infra-red spectroscopy of (a, b) tricalcium silicate and 20% zirconium oxide (c, d) Bioaggregate; (e, f) Biodentine; (g, h) intermediate restorative material (IRM); (CS, tricalcium silicate; C-S-H, calcium silicate hydrate; CC, calcium carbonate; OH, hydroxyl groups; P, Portlandite; T, Tantalum oxide; Z, zirconium oxide; Zn, zinc oxide).

was necessary to achieve a radiopacity similar to that of ProRoot MTA (Cutajar *et al.* 2011). Thus, Biodentine would be expected to have a lower radiopacity value.

Intermediate Restorative Material (IRM) was composed of zinc oxide and a phase rich in carbon. The phase rich in carbon could signify the presence of a hydrocarbon-based material because organic materials

only show a carbon peak on X-ray energy dispersive analysis. The poly-methyl methacrylate was not identified on XRD as X-ray diffraction analysis only displays crystalline structures. However, the FT-IR plot of IRM demonstrated the typical presence of C-O and C-H bonds. Eugenol, which is mixed with IRM, is also organic and contains alcohol and methoxy groups that were detected on the FT-IR scan. There were very large areas of eugenol present in the set material as evident from the scanning electron micrographs.

On hydration, the tricalcium silicate cement-based materials formed calcium silicate hydrate and calcium hydroxide. The FT-IR plots indicated the presence of calcium silicate hydrate by the typical bands present between 1200 and 800 cm^{-1}. Unhydrated tricalcium silicate also exhibits bands in the 1200–800 cm^{-1} region also attributed to the asymmetrical and symmetrical stretching vibrations of Si-O. However, the peaks for calcium silicate hydrate are broader and not so well defined, indicating the presence of a poorly crystalline solid.

The formation of calcium hydroxide was evident from the Portlandite peak on X-ray diffraction analysis. This is in accordance with previous reports on the hydrating of tricalcium silicate cement (Camilleri 2011) and Bioaggregate (Park *et al.* 2010). The calcium hydroxide released in solution was detected in the calcium ion analysis of the leachate. All tricalcium silicate-based cements exhibited calcium ions on leachate analysis and a resultant alkaline pH, which rose after 1 day of immersion in solution and was maintained throughout the 28-day period. The presence of O-H groups in FT-IR plots was shown in the region of 1640 cm^{-1}. The peaks in this area are characterized by noise due to the presence of stretching vibrations of physically adsorbed water. The portlandite peak and the calcium ion release in solution were not manifested in IRM.

The Bioaggregate exhibited a phosphorus peak in the EDX analysis, but the phosphorus-containing phase was not visible in the scanning electron micrographs of the set cement. Biodentine incorporated additives to both the powder and the liquid phase. The Biodentine liquid included a chloride-containing phase (Camilleri *et al.* 2012). The Biodentine powder had inclusions of calcium carbonate. The calcium carbonate particles were large compared to the cement particles. They also exhibited hydration product around their circumference. Calcium carbonate is added by the manufacturer to act as a nucleation site. Although it is not cementitious as it does not react

with water to form reaction by-product; however, it allows the formation of reaction rims around it, thus enhancing the hydration and producing a denser microstructure. The presence of a carbonate phase was verified by XRD and FT-IR analysis. The prototype cement and Bioaggregate also showed CO_3^{3-} stretching; however, this could be attributed to surface carbonation of the specimens by reaction of the calcium hydroxide with atmospheric carbon dioxide (Pointeau *et al.* 2001).

Although the materials were immersed in a physiological solution, its presence was not detected in the XRD plots and FT-IR analysis. The presence of hydroxyapatite on calcium silicate-based cements has been demonstrated previously (Tay *et al.* 2007, Taddei *et al.* 2009, Han *et al.* 2010). This hydroxyapatite was present on the material surface and was implicated in the bioactivity potential of calcium silicate-based cements. The testing executed in the current research was performed on powdered samples rather than on the material surface. Testing-powdered specimens ensure the proper characterization of the materials. The calcium phosphate phase exhibits the strongest peak at 32.052° 2θ on XRD analysis. This peak coincides with the tricalcium silicate peak for all the tricalcium silicate-based cements and zirconium oxide peak for the radiopacified tricalcium silicate cement and Biodentine. Characteristic vibration modes of PO_4^{3-} group (565, 599, 960 and 1041 cm^{-1}) and to CO_3^{2-} group (871, 1414 and 1454 cm^{-1}) of apatite in FT-IR analysis were not observed in this study.

Conclusions

The hydration of a prototype-radiopacified tricalcium silicate cement, Biodentine and Bioaggregate resulted in the formation of calcium silicate hydrate and calcium hydroxide, which was leached in solution. The hydrated materials were composed of a cementitous phase, which was rich in calcium and silicon and a radiopacifying material. Biodentine and Bioaggregate included additives in the powders. IRM was composed of zinc oxide interspersed in a matrix of organic material; it showed no evidence of leaching of calcium ions in solution.

Acknowledgements

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