The microstructure and surface morphology of radiopaque tricalcium silicate cement exposed to different curing conditions

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ABSTRACT

Objective. Tricalcium silicate is the major constituent phase in mineral trioxide aggregate (MTA). It is thus postulated that pure tricalcium silicate can replace the Portland cement component of MTA. The aim of this research was to evaluate the microstructure and surface characteristics of radiopaque tricalcium silicate cement exposed to different curing conditions namely at 100% humidity or immersed in either water or a simulated body fluid at 37 °C.

Methods. The materials under study included tricalcium silicate and Portland cements with and without the addition of bismuth oxide radiopacifier. Material characterization was performed on hydrated cements using a combination of scanning electron microscopy (SEM) with X-ray energy dispersive (EDX) analyses and X-ray diffraction (XRD) analyses. Surface morphology was further investigated using optical profilometry. Testing was performed on cements cured at 100% humidity or immersed in either water or Hank's balanced salt solution (HBSS) for 1 and 28 days at 37 °C. In addition leachate analysis was performed by X-ray fluorescence of the storage solution. The pH of the storage solution was assessed.

Results. All the cements produced calcium silicate hydrate and calcium hydroxide on hydration. Tricalcium silicate showed a higher reaction rate than Portland cement and addition of bismuth oxide seemed to also increase the rate of reaction with more calcium silicate hydrate and calcium hydroxide being produced as demonstrated by SEM and XRD analysis and also by surface deposits viewed by the optical profilometer. Cement immersion in HBSS resulted in the deposition of calcium phosphate during the early stages following immersion and extensive calcification after 28 days. The pH of all storage solutions was alkaline. The immersion in distilled water resulted in a higher pH of the solution than when the cements were immersed in HBSS. Leachate analysis demonstrated high calcium levels in all cements tested with higher levels in tricalcium silicate and bismuth replaced cements.

Significance. Tricalcium silicate cement is more bioactive than Portland cement as demonstrated by various characterization techniques. The bioactivity was monitored by measuring the production of calcium hydroxide and the formation of calcium phosphate when in contact with simulated body fluids.

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1. Introduction

Tricalcium silicate (TCS) is one of the main constituents of mineral trioxide aggregate (MTA). It constitutes approximately 52% of the un-hydrated material [1,2]. Portland cement which is the main constituent of MTA is in turn made up of 67–75% of tricalcium silicate [1,3]. This percentage composition differs depending on the type of cement and the manufacturer [4]. It is thus hypothesized that pure tricalcium silicate will be a candidate to replace the Portland cement component of MTA. Both MTA and tricalcium silicate cement belong to a new class of dental materials namely the Hydraulic Silicate Cements [5].

Hydration of tricalcium silicate in Portland cement results in the formation of calcium silicate hydrate gel, calcium hydroxide and un-reacted tricalcium silicate [6]. This analysis was confirmed by research performed on Portland cement intended for use as a dental material whereby after 28 days of hydration a small fraction of the tricalcium silicate in Portland cement remained unreacted on hydration, and the rest formed calcium silicate hydrate and calcium hydroxide [1,3]. In Portland cement the calcium silicate hydrate and calcium hydroxide are also produced by the reaction of dicalcium silicate with water [6]. Pure tricalcium silicate also reacts with water producing calcium silicate hydrate and calcium hydroxide leaving a small portion of un-reacted material [1,3,7]. The calcium hydroxide produced from the tricalcium silicate hydration possesses antibacterial and anti-inflammatory properties [8] mainly due to the high (alkaline) pH of the surrounding environment after it dissolves [9]. Calcium hydroxide can further induce mineralization which results in repair of damaged dentin matrix [10].

Pure tricalcium silicate is prepared by the sol-gel method [11]. Tetraethyl Orthosilicate (TEOS, $Si(OC_2H_5)_4$) and nitric acid as catalyst are combined in water under continuous stirring. Calcium Nitrate ($Ca(NO_3)_2 \cdot 4H_2O$) is then added and the solution is maintained at $60\,^\circ\mathrm{C}$ until gelation occurs. The gel is then dried at $120\,^\circ\mathrm{C}$ and calcined for 8 h at $1450\,^\circ\mathrm{C}$. The resultant powder is finally ground and sieved. The advantage of this method is that the raw materials are pure, unlike those used in Portland cement manufacture. In addition the raw materials are free of aluminum which has been linked to Parkinson's and Alzheimer's disease [12]. Tricalcium silicate cement is nowadays used as the main constituent of a number of proprietary brands namely Biodentine (Active Biosilicate Technology™, Septodont, Saint-Maur-des-Fossés Cedex, France) and Bioaggregate (Verio Dental, Vancouver, Canada).

Both MTA and Portland cement have demonstrated bioactivity, i.e. both form a bone-like hydroxyapatite layer on the surface when immersed in physiological solution; this is a common characteristic observed in calcium-silicate based materials [13–16]. This layer provides the benefits of increasing the sealing ability of MTA and promoting remineralization and regeneration of hard tissues [13]. Tricalcium silicate also exhibits the ability to form hydroxyapatite in contact with simulated body fluid. This layer is also instrumental in maintaining the bone–biomaterial interface when implanted in the body [7]. Furthermore, calcium silicate cements including tricalcium silicate were found to have advantageously shortened setting time compared with MTA, and postulated to be suitable for replacement of the cement component of MTA due to their similar composition and bioactivity [17]. Tricalcium silicate cement possesses good injectability, good bioactivity and moderate in vitro degradability, meaning that ultimately the body may be able to replace the implanted cement by natural tissue [7]. The addition of up to 30% calcium carbonate ($CaCO_3$), calcium sulphate ($CaSO_4 \cdot 0.5H_2O$) and calcium chloride resulted in improvement in physical properties of tricalcium silicate cement [7,8,18] as well as enhancing the bioactivity and degradability of the resultant composite material [18]. Tricalcium silicate exhibited a similar cell mortality and mutagenicity to that of MTA [19]. It was thus concluded that tricalcium silicate was safe to use as a bulk restorative material, without requiring any surface treatments, as an alternative to traditional materials [19,20].

The aim of this research was to evaluate the microstructure and surface characteristics of radiopaque tricalcium silicate cement exposed to different curing conditions namely at 100% humidity or immersed in either water or a simulated body fluid at $37\,^\circ\mathrm{C}$.

2. Materials and methods

Materials used in this study were Portland cement (PC; CEM 1, 52.5 N; LaFarge Cement, Birmingham, UK), tricalcium silicate (TCS manufactured using the sol-gel method; Mineral Research Processing, Meyzieu, France), and bismuth (III) oxide, Bi_2O_3 ($10\,\mu m$ 223891-100G – Sigma Aldrich, St. Louis, MO, USA). The prototype materials and their corresponding water/powder ratios for each mixture are shown in Table 1. The bismuth oxide replaced the 20% of the cement by weight.

2.1. Microscopy and elemental analysis of powders and hydrated cements

Cylindrical specimens with 10 ± 0.1 mm diameter and a height of 2 ± 0.1 mm were prepared. The resultant discs were incubated in an atmosphere with 100% relative humidity for 24 h at $37 \pm 1\,^\circ\mathrm{C}$ to allow the cement to cure. They were then removed from the molds and either allowed to cure at $37 \pm 1\,^\circ\mathrm{C}$ in an atmosphere of 100% relative humidity, or immersed in either distilled water or Hank's balanced salt solution (HBSS; H6648, Sigma Aldrich, St. Louis, MO, USA). The composition of the HBSS was (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.

After both 1 day and 28 days of curing, the specimens were taken out of solution, dried using a filter paper and placed in an oven at around $60\,^\circ\mathrm{C}$ for 24 h to dry. They were then placed overnight in a vacuum chamber and subsequently mounted on aluminum stubs. The cements were then examined under the scanning electron microscope (SEM; Zeiss MERLIN Field Emission SEM, Carl Zeiss NTS GmbH, Oberkochen, Germany) and micro-structural analysis was performed.

2.2. X-ray diffraction (XRD) analysis

Phase analysis was carried out on the cements using X-ray diffraction (Bruker D8 Advance, Bruker Corp., Billerica, MA, USA) in locked mode. The diffractometer was set with a Mo Kα

Table 1 – Test materials corresponding water/powder ratios.				
Material	Acronym	Cement proportion (%)	Radiopacifier proportion (%)	Water/powder ratio
Portland cement	PC	100	0	0.30
Portland cement and bismuth oxide	PCB	80	20	0.30
Tricalcium silicate	TCS	100	0	0.37
Tricalcium silicate and bismuth oxide	TCSB	80	20	0.37

radiation tube. The operating current and voltage was set at 35 mA and 45 kV respectively. Specimens were presented both as solid discs of diameter 10 ± 0.1 mm and height 2 ± 0.1 mm for surface analysis, and in powdered form. The intensity of X-rays from the sample were measured using a detector which was rotated between 5° and 25°. A step of 0.01° 2θ and a step time of 0.4 s was used. For the powdered specimens the sample holder was spun at 15 rpm. Analysis was performed on cement samples following 1 day and 28 days of curing at 37 ± 1 °C in an atmosphere of 100% relative humidity, or immersed in either distilled water or HBSS. Phase identification was accomplished using a search-match software utilizing ICDD database (International Center for Diffraction Data, Newtown Square, PA, USA).

2.3. Evaluation of pH of leachate

Discs of diameter 15 ± 1 mm and a thickness of 1 ± 0.1 mm were prepared of each cement type. After storage in an incubator at 37 ± 1 °C for 24 h and removal from the molds, the materials were immersed upright in either 10 ml water or Hanks balanced salt solution. The pH readings of the storage solution were taken using a pH meter (Hanna HI 9811, Hanna Instruments, Woonsocket, RI, USA) prior to immersion and after 1, 7, 14, 21 and 28 days post-immersion. Three replicate samples of each of the four materials were made for each of the two solutions (24 disks in total).

2.4. Chemical analysis of leachate

The chemical analysis of the cement products released in water and HBSS was performed using Energy Dispersive X-ray fluorescence (EDXRF). One disk of each of the four materials was immersed in 5 ± 0.001 ml of either distilled water or HBSS in an airtight polycarbonate container, for a period of either 1 day or 28 days from the immersion, at a constant 37 ± 1 °C. At each time point the disks were removed from solution and the solution was analyzed using EDXRF (Bruker S2 Ranger, Bruker Corporation, Madison, USA) using water as matrix and 4 µm liquid prolene film. Containers filled with water and HBSS were used as controls.

2.5. Profilometry

Surface analysis of pre-prepared samples was carried out using 3D optical profilometry. The profiler (Xyris 2000WL, TaiCaan Technologies, Southampton, UK) used a precision motion system and a confocal polychromatic (white) light optical probe to measure the displacement at the cement surface over a specified area. The region measured was a 1.000 mm by 1.000 mm area at the center of each cement sample with a measurement taken every 2.5 µm in the X and Y

axes. This provided a 3D map of the cement surface consisting of 160,801 evenly spaced samples. Each measurement took approximately 12 min. Measurements of the cement samples were taken before (Day 0) and after (Day 28) either immersion in distilled water, immersion in HBSS or exposure to ambient atmosphere. The measurements were then analyzed for the changes in areal roughness, and 3D surface area.

2.6. Statistical analysis

The data were evaluated using SPSS (Statistical Package for the Social Sciences) software (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 post hoc test were used to perform multiple comparison tests.

3. Results

3.1. Microscopy and elemental analysis of powders and hydrated cements

The scanning electron micrographs for the cements cured for 1 and 28 days in different environments are shown Fig. 1. Both tricalcium silicate and Portland cement exhibited the formation of calcium silicate hydrate which had honeycomb morphology (Fig. 1a) for all curing conditions. The Portland cement specimens also exhibited the deposition of ettringite needle-like crystals over the cement surface (Fig. 1b).

Calcium hydroxide (hexagonal crystals) was deposited over both tricalcium silicate (Fig. 1c) and Portland cement (Fig. 1d) when exposed to 100% atmospheric humidity conditions. Higher amounts of calcium hydroxide were observed on tricalcium silicate cement than on Portland cement. The calcium hydroxide was identified by EDX analysis (Fig. 1e). Immersion of all cement types in HBSS for 1 day resulted in the deposition of calcium phosphate over the cement surfaces (Fig. 1f). The EDS spectrum is shown in Fig. 1g. The cements that were cured in HBSS for 28 days had an extensive deposition of calcium carbonate with different crystal morphology over the cement surface (Fig. 1h–k).

3.2. X-ray diffraction (XRD) analysis

The X-ray diffraction analysis of the cured cements in powdered form after 1 day and 28 days of hydration is shown in Fig. 2. The Portlandite peak (CH; ICDD number: 44-1481) at 8.2° 2θ was evident in all 1 day specimens. The Portlandite peak intensity was not evident in the cements containing bismuth oxide after 28 days of curing. All the specimens containing bismuth oxide as radiopacifier exhibited a peak for bismuth oxide (BO; ICDD number: 41-1449) at 12.6° 2θ. The tricalcium
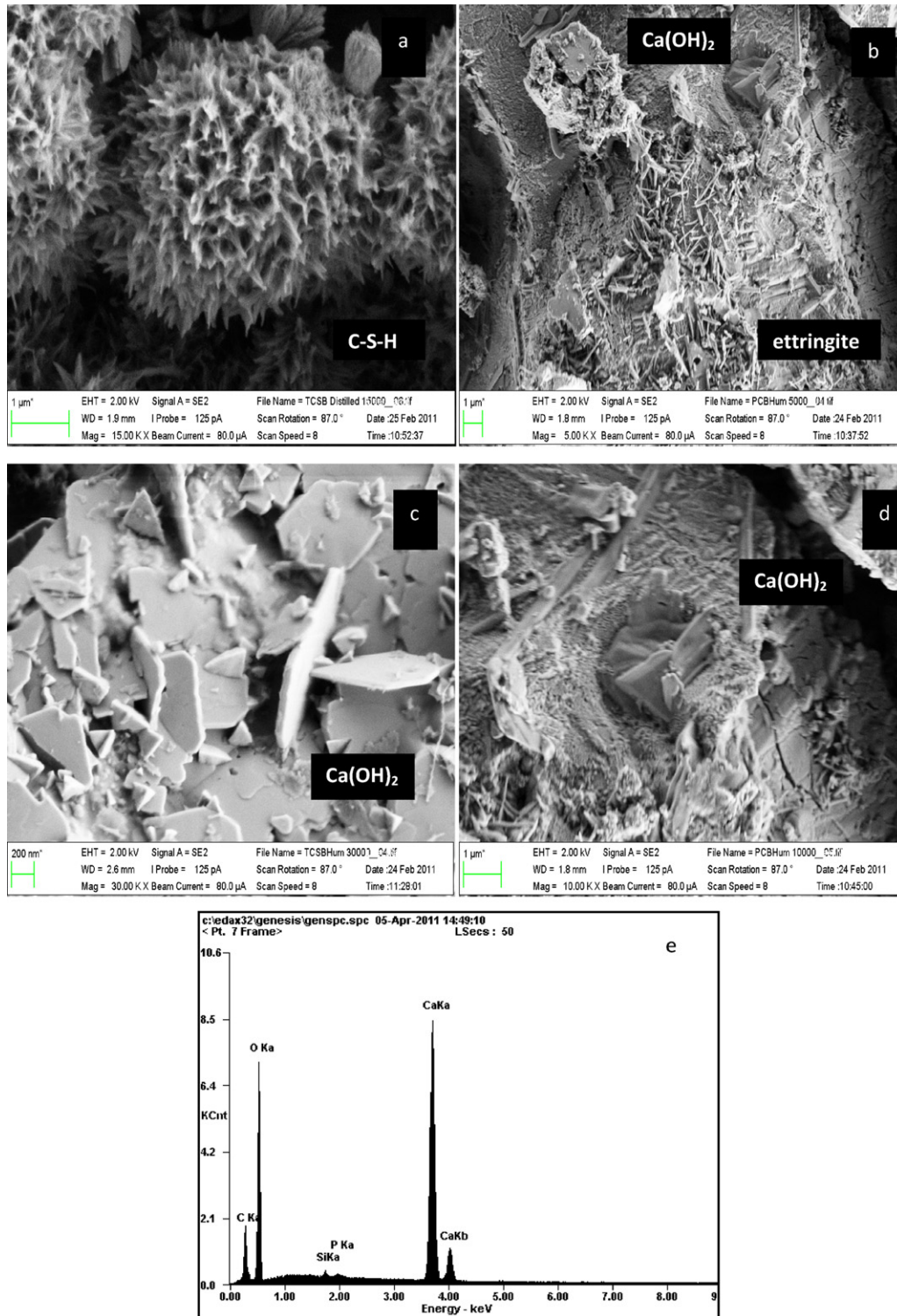
Fig. 1 – Scanning electron micrographs of cements cured for 1 day in different environments showing (a) C–S–H; (b) ettringite deposition over Portland cement grains; calcium hydroxide deposits on (c) tricalcium silicate and (d) Portland cement; (e) EDX spectrum of calcium hydroxide crystals (f) calcium phosphate deposited over cements cured in HBSS after 1 day; (g) EDX spectrum of calcium phosphate; (h–k) surface carbonation over cements curried in HBSS for 28 days.
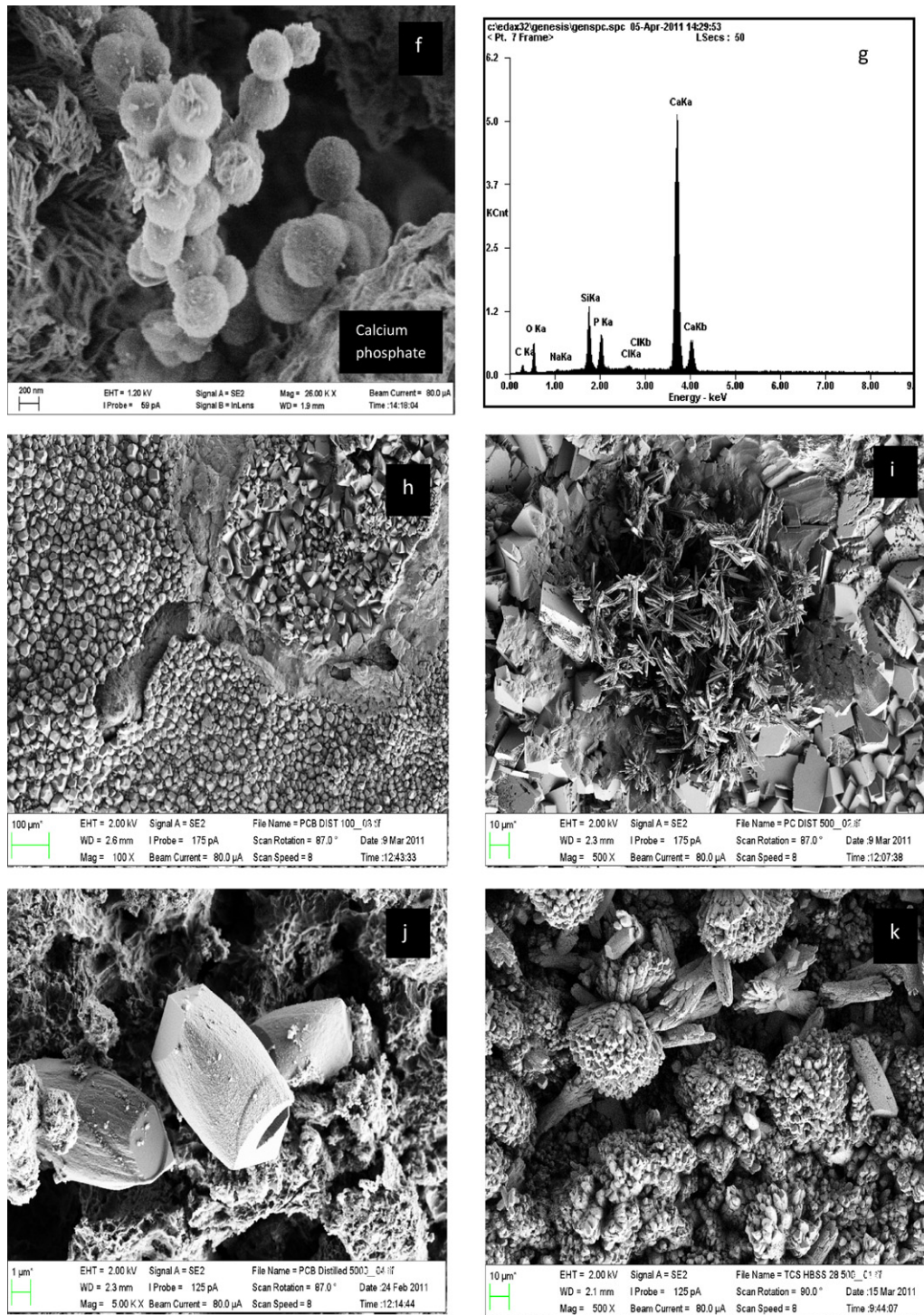
Fig. 1 – (continued)

silicate peaks (C_3S; ICDD number: 31-0301) were lower at 28 days when compared to the 1 day tricalcium silicate peaks for the bismuth oxide replaced cements.

The surface scans of the cements are shown in Fig. 3. The 1 day scans exhibited the tricalcium silicate peaks (C_3S; ICDD number: 31-0301) at 13.4, 14.6 and 15.5° 2θ and the bismuth oxide peak (BO; ICDD number: 41-1449) in the bismuth oxide replaced cements at 12.6° 2θ. After 28 days the surface scans were mostly flat with the cements cured in HBSS exhibiting a strong peak at 13.5° and a smaller peak at 21.5° 2θ, indicating the presence of calcite (CC; ICDD number: 03-0569).

3.3. Evaluation of pH of leachate

The results for the pH readings of the storage solution are shown in Fig. 4. In distilled water (Fig. 4a), the pH of all four
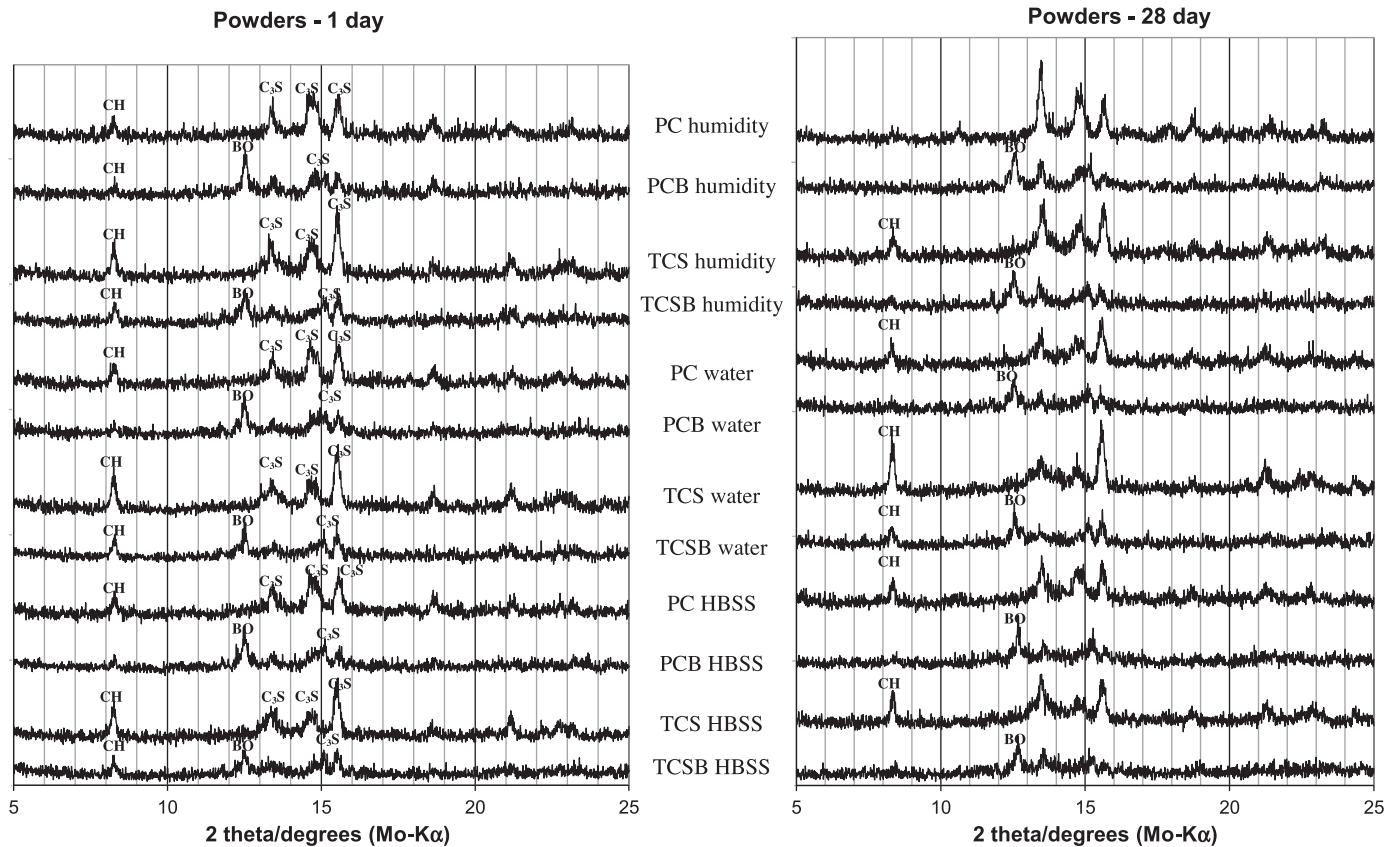
Powders - 1 day

Powders - 28 day

Fig. 2 – X-ray diffractograms of the powdered cements cured at either 100% humidity or immersed in distilled water or HBSS after 1 and 28 days (CH: calcium hydroxide, BO: bismuth oxide, C$_3$S: tricalcium silicate).

materials was similar to that of the HBSS solution (Fig. 4b) at 1 day ($P > 0.05$) but higher for all time periods thereafter ($P < 0.05$). There was no statistically significant difference between the materials between Days 1 and 21 ($P > 0.05$) when the cements were immersed in distilled water. At 28 days the tricalcium silicate, bismuth replaced Portland cement and bismuth oxide replaced tricalcium silicate were more alkaline than the Portland cement ($P = 0.002$, $P = 0.009$, $P = 0.001$ respectively). The pH was alkaline for all four materials at all time periods when the cements were immersed in HBSS. There was no statistically significant difference ($P > 0.05$) between the pH of the four materials at all time periods.

3.4. Chemical analysis of leachate

The leachate was analyzed for bismuth, calcium, chlorine and phosphorus ion release in water and HBSS after 1 and 28 days (Table 2). All materials leached calcium for all time periods in both soaking solutions. The tricalcium silicate-based cements (TCS and TCSB) leached more calcium than the Portland cement-based materials (PC and PCB) in distilled water at both 1-day and 28-days, and in HBSS after 28 days. The addition of bismuth oxide in PCB and TCSB generally increased the leaching of calcium for both Portland cement and tricalcium silicate cement. More calcium was leached in HBSS when compared to the calcium present in distilled water.

Bismuth was detected only in the cements to which bismuth oxide was added as a radiopacifier. There was more bismuth present in HBSS than in water and the leaching was higher after 28 days. The phosphorus was present at 1 day in the storage solutions composed of HBSS. It could not be detected at 28 days. On the other hand chlorine was absorbed from the HBSS solution at 1 day resulting in negative values thus indicating that the leachates at 1 day contained less chlorine than the blank solution. However at 28 days chlorine was present in the storage solutions of all the cements immersed in HBSS.

3.5. Profilometry

The results for the surface profilometric analysis for the four cements cured at different curing conditions for 28 days are shown in Fig. 5. The numerical data is shown in Table 3. Marked surface changes were observed by all cement types when immersed in water. The TCS and TCSB samples showed a greater number and greater size of surface feature that the PC and PCB samples. A number of very large features (0.6–0.8 mm in height) were observed on the TCS and TCSB samples. When examined these were found to be hollow. A slightly greater surface change was observed in the samples with bismuth oxide. All surfaces left to cure in atmospheric conditions showed a very similar decrease in surface roughness and 3D surface
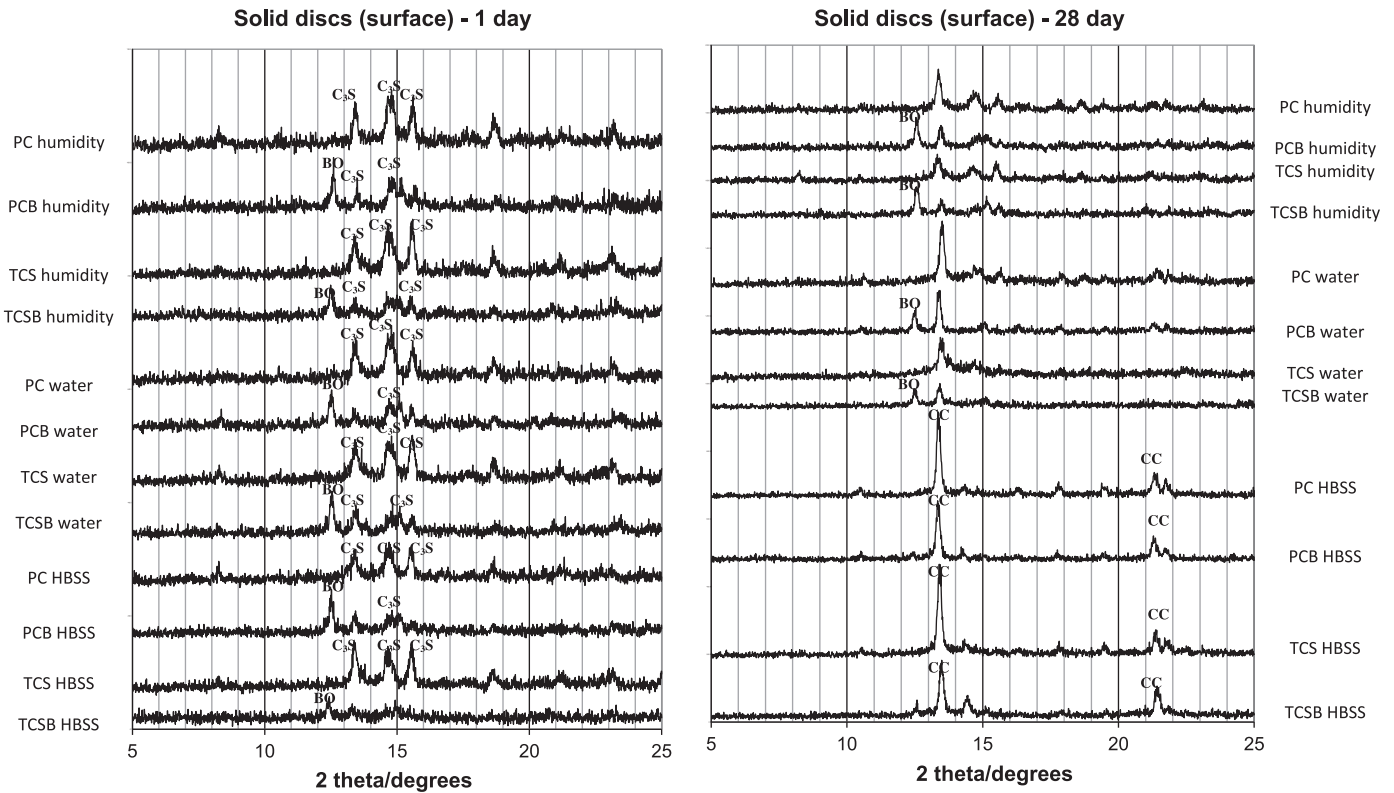
Fig. 3 – X-ray diffractograms of surfaces of cements cured at either 100% humidity or immersed in distilled water or HBSS after 1 and 28 days (CC: calcium carbonate, BO: bismuth oxide C_3S: tricalcium silicate).

area indicating a smoothing of the surface. The same trend was observed in the test samples soaked in HBSS with the exception of the TCS without bismuth oxide which showed a significant increase in surface area and roughness.

4. Discussion

Tricalcium silicate is one of the main constituents of MTA. In this study pure tricalcium silicate was investigated to establish its suitability as a replacement for the Portland cement component of MTA. Material characterization was performed

using a combination of scanning electron microscopy and X-ray energy dispersive (EDX) analysis. EDX allows material surface visualization and characterization giving qualitative information of the elemental constitution of the test materials. In addition X-ray diffraction (XRD) was also performed both on powdered specimens and on solid samples thus determining the particular phases present in each material. A graph of intensity against 2θ is plotted and as the particular peaks on this graph are unique to each material, they can thus be used to identify the phases present in the specimen. Because XRD relies on diffraction, it can only identify phases with a regular (crystalline) structure. Phases with a random (i.e. amorphous)

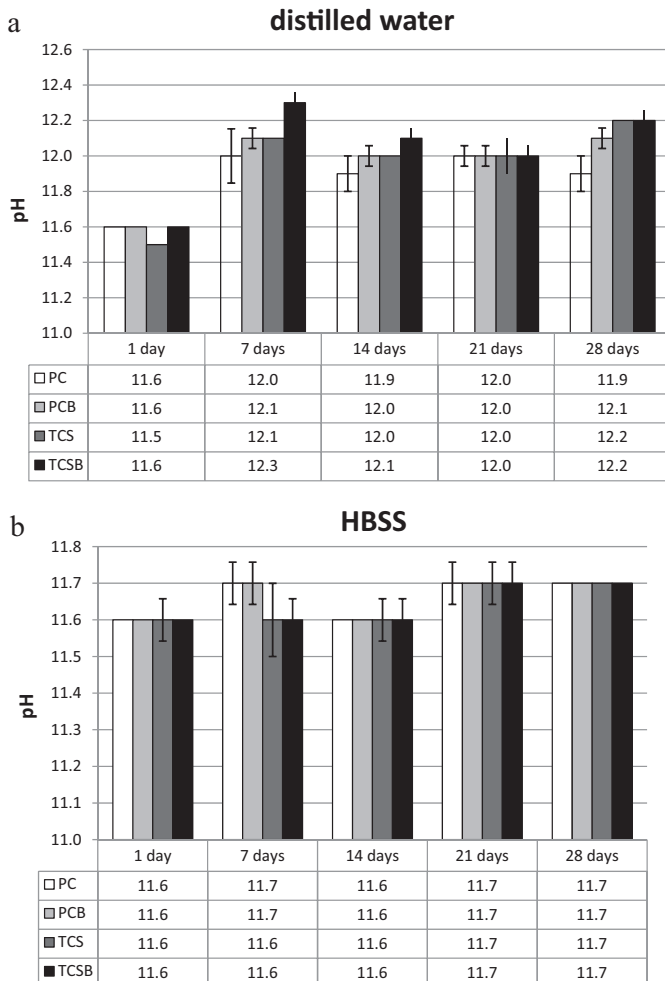| Table 2 – Elements leached in distilled water and HBSS after 1 day and 28 days (ppm). | | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Distilled water | | | | HBSS | | | |
| | PC | PCB | TCS | TCSB | PC | PCB | TCS | TCSB |
| **1 day** | | | | | | | | |
| Bi | 0 | 0 | 0 | 0 | 0 | 41 | 0 | 21 |
| Ca | 682 | 952 | 962 | 1782 | 2727 | 5337 | 2547 | 1657 |
| Cl | 38 | 25 | 25 | 15 | −440 | −330 | −260 | −170 |
| P | 0 | 0 | 0 | 0 | 277 | 2020 | 394 | 453 |
| **28 days** | | | | | | | | |
| Bi | 0 | 11 | 0 | 10 | 0 | 89 | 0 | 151 |
| Ca | 1222 | 1442 | 1662 | 1702 | 1002 | 3472 | 5262 | 7262 |
| Cl | 21 | 42 | 19 | 22 | 4751 | 4791 | 4941 | 5071 |
| P | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 542 |
| PC: Portland cement, PCB: Portland cement and 20% bismuth oxide, TCS: tricalcium silicate, TCSB: tricalcium silicate and 20% bismuth oxide. | | | | | | | | |

Table 3 – Profilometry data for Roughness and 3D surface area before and after material soaking.			
	Cure in atmosphere	Immersion in D H$_2$O	Immersion in HBSS
TCS			
Roughness average Day 0 [Sa[a]] (µm)	3.52	9.48	6.33
Roughness average Day 28 [Sa[a]] (µm)	1.89	26.36	10.72
3D surface area Day 0 (mm^2)	1.14	1.45	1.11
3D surface area Day 28 (mm^2)	1.02	2.66	2.15
TCSB			
Roughness average Day 0 [Sa[a]] (µm)	2.69	4.58	10.36
Roughness average Day 28 [Sa[a]] (µm)	1.98	76.56	7.57
3D surface area Day 0 (mm^2)	1.16	1.25	1.36
3D surface area Day 28 (mm^2)	1.07	4.24	1.26
PC			
Roughness average Day 0 [Sa[a]] (µm)	14.06	5.14	12.36
Roughness average Day 28 [Sa[a]] (µm)	9.96	13.01	7.54
3D surface area Day 0 (mm^2)	1.59	1.09	1.58
3D surface area Day 28 (mm^2)	1.21	2.06	1.41
PCB			
Roughness average Day 0 [Sa[a]] (µm)	3.94	4.69	7.45
Roughness average Day 28 [Sa[a]] (µm)	3.43	20.11	4.37
3D surface area Day 0 (mm^2)	1.32	1.39	1.54
3D surface area Day 28 (mm^2)	1.15	2.31	1.31
[a] Sa applied using a 0.8 mm Gaussian Filter.			

a **distilled water**

	1 day	7 days	14 days	21 days	28 days
PC	11.6	12.0	11.9	12.0	11.9
PCB	11.6	12.1	12.0	12.0	12.1
TCS	11.5	12.1	12.0	12.0	12.2
TCSB	11.6	12.3	12.1	12.0	12.2

b **HBSS**

	1 day	7 days	14 days	21 days	28 days
PC	11.6	11.7	11.6	11.7	11.7
PCB	11.6	11.7	11.6	11.7	11.7
TCS	11.6	11.6	11.6	11.7	11.7
TCSB	11.6	11.6	11.6	11.7	11.7

structure cannot be identified by XRD. XRD is useful in performing chemical analyses of cement and related materials since Portland cement, tricalcium silicate and bismuth oxide, are crystalline. The precipitated compounds, including calcium hydroxide, calcium phosphate and calcium carbonate, are also crystalline.

Leachate analysis was carried out using X-ray fluorescence (XRF). Measurement of the radiation emitted from the sample and separating by wavelength, the constituent elements of the sample could be deduced by comparing with known energy levels for each element [21]. The main advantages of XRF are that no sample preparation is required and it is non-destructive. Because the sample is not modified in any way before or during analysis there is no opportunity for unwanted changes to the sample to take place. Another potential method for carrying out the leachate analysis is ICP (inductively coupled plasma), which has the potential to be more accurate [22], but requires vaporising the sample with an argon plasma torch [23].

While the profilometry data showed clear trends in the samples there is scope for a better quantitative analysis of the surface changes that are occurring. Firstly the size and distribution of the largest features observed on the TCS and TCSB samples after immersion in water (in cases several millimeters across) had not been anticipated and thus the area selected for measurement was not sufficiently large to provide a representative sample of the surface. Care was taken in choosing a representative area of the surface but a larger measured area

Fig. 4 – Variation of pH of specimens immersed in (a) water and (b) HBSS at 37 °C, measured at 7-day intervals over a 28-day period (±SD).
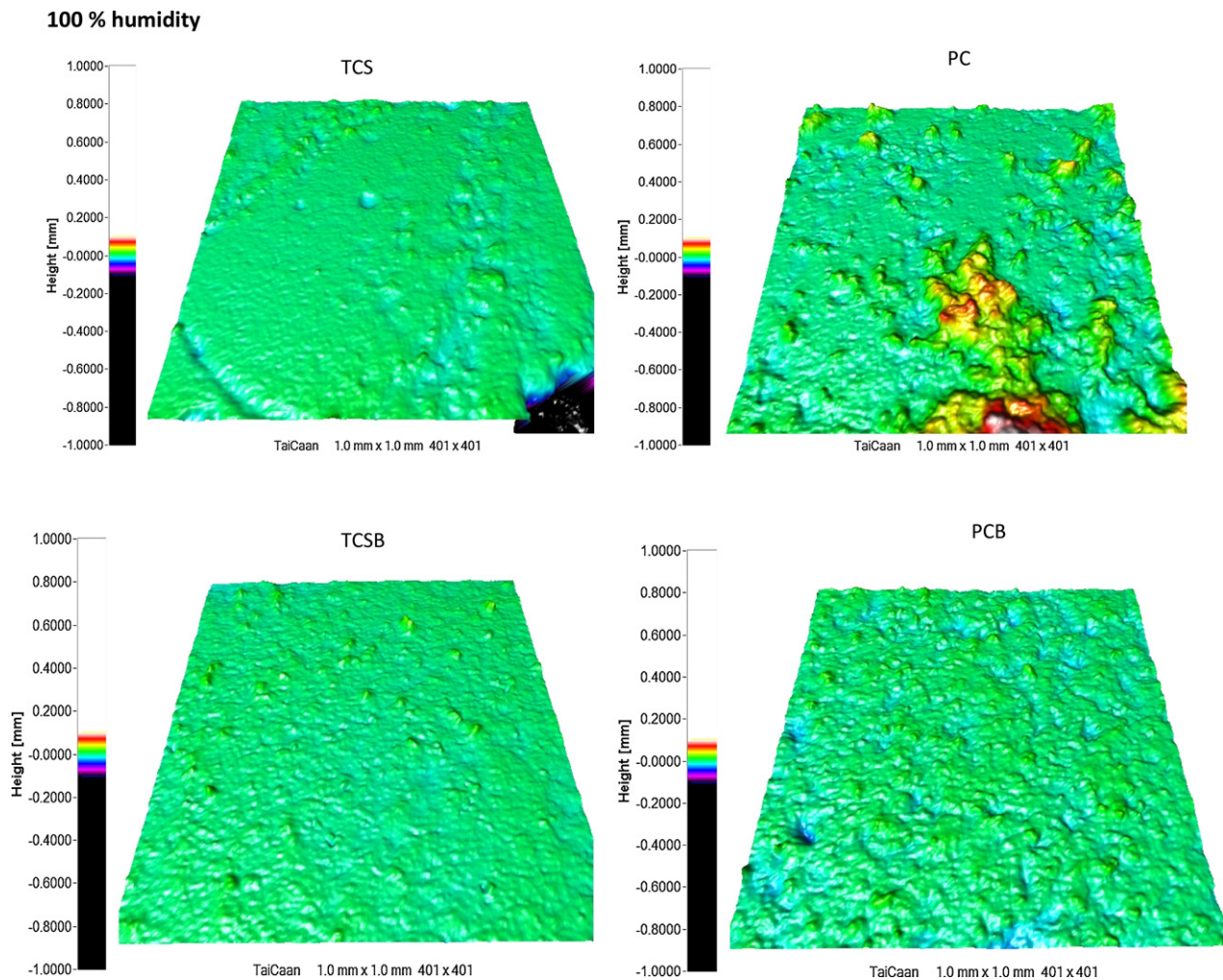
100 % humidity

Fig. 5 – Surface characteristics measured by optical profilometer of tricalcium silicate cement (TCS), Portland cement (PC), and the radiopacified cements (TCSB, PCB) after 28 days exposed to different environmental conditions.

would have provided higher quality quantitative data. Secondly, while the data showed a clear trend of predominantly depositional process, in several cases it was clear that both erosional and depositional processes had occurred (particularly the HBSS soaked samples) and had completely changed the original surface. By applying a datum to the samples it would be possible to measure volume change thus quantitatively evaluating these processes.

The results indicate that all four materials were alkaline for all time periods. This alkalinity is a result of the calcium hydroxide produced during hydration. In solution calcium hydroxide liberates hydroxide ions when it dissociates into Ca and OH ions. Both major constituents of Portland cement, namely dicalcium silicate and tricalcium silicate, liberate calcium hydroxide as a reaction by-product upon hydration [4,6]. Tricalcium silicate cement reacts in a similar fashion with the production of calcium hydroxide on hydration and resulting in an alkaline pH. The pH level remained stable over time at a value of around 11–12 which is in agreement with the results reported in literature for both tricalcium silicate cement [7,17] and MTA [24,25].

The release of calcium ions from all the test materials was further demonstrated by the EDXRF results on the leachate. Calcium ions were released from all the materials at all time periods and in both distilled water and physiological solution. The calcium ion release was generally greater in HBSS than in distilled water and calcium ion concentration of tricalcium silicate-based materials increased after 28 days as opposed to that of Portland cement and related materials. This is in accordance to previous research which demonstrated a higher release of calcium in HBSS than in water, and a higher calcium ion release in tricalcium silicate cement than Portland cement [3]. The higher calcium ion release of the tricalcium silicate cements and the bismuth oxide replaced cements was verified by XRD analysis and by optical profilometry where the surface deposits were shown to be thicker. The cements soaked in HBSS did not show surface deposits when analyzed by optical profilometry as would have been expected. Since the calcium release is the reason why the material is bioactive [13–16], this suggests that tricalcium silicate will be more bioactive with time and is therefore superior to a Portland-cement based material. The results are in accordance to those
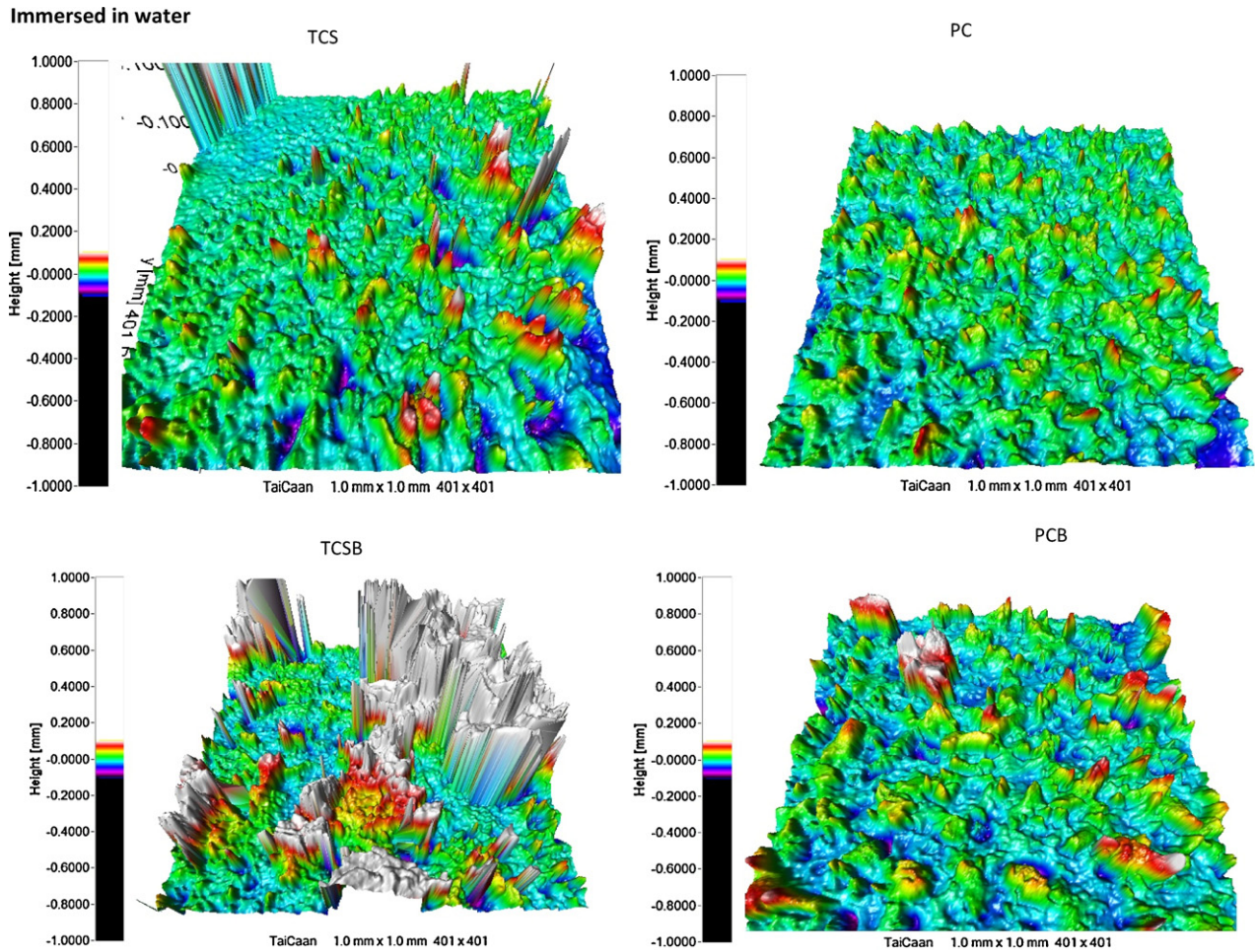
Immersed in water

Fig. 5 – (continued)

published in other studies on MTA [1,24] and tricalcium silicate [7]. The amount of bismuth oxide leached in solution was minimal and not significantly different between the two materials.

The products of hydration, namely calcium silicate hydrate and calcium hydroxide, were clearly visible on the cement surface using the scanning electron microscope. In the case of Portland cement, ettringite crystals were also identified. The precipitate of calcium phosphate, thought to be the cause of the cement's bioactivity was also observed on both tricalcium silicate and Portland cement. The phosphate in the HBSS reacts with the calcium hydroxide from the cement to form hydroxyapatite [13–16,27]. The hydroxyapatite was identified by scanning electron microscopy and EDX analysis. The observed structures are in agreement with those reported in literature [13,14,26–28]. The reaction of the phosphate from the simulated body fluid to calcium hydroxide released from the cement was further demonstrated by the depletion of the phosphate ions in solution which was evident for all cement types and more enhanced at 28 days where no phosphorus was present in solution. This has been previously reported for tricalcium silicate cement and Portland cements replaced with zirconium oxide [3,25].

Calcium carbonate was found on the surface of the specimens that were immersed in HBSS for 28 days. The calcium carbonate deposition was evident on the scanning electron micrographs and also on the XRD scans. Performing XRD scans on material surfaces can lead to erroneous results whereby the chemical composition of the material under study can be wrongly interpreted by the analysis of the surface deposits only. The calcium carbonate may have formed from the reaction of calcium hydroxide with carbonates. The main source of carbonate in HBSS is the sodium hydrogen carbonate added to act as a buffer (to ensure the correct pH). The second source is atmospheric carbon dioxide which naturally dissolves in water to form carbonic acid. The calcium carbonate produced had various morphologies. The morphology of calcium carbonate can vary depending on the ambient conditions present during the crystal synthesis namely the pressure [29] temperature [30], pH [31] and presence of other chemicals in the electrolyte [32]. In vivo the surface carbonation may very likely occur as well. Atmospheric carbon dioxide may not play a role in vivo however both blood plasma and synthetic tissue fluids contain sodium hydrogen carbonate [33] which may contribute to the formation of calcium carbonate on the cement surface. The presence of carbonated apatite synthesized by the interaction of phosphate containing fluids with MTA and Portland cement has already been reported both in the presence of synthetic tissue fluids [15,16,27] and also in contact with blood plasma in animal studies [34].

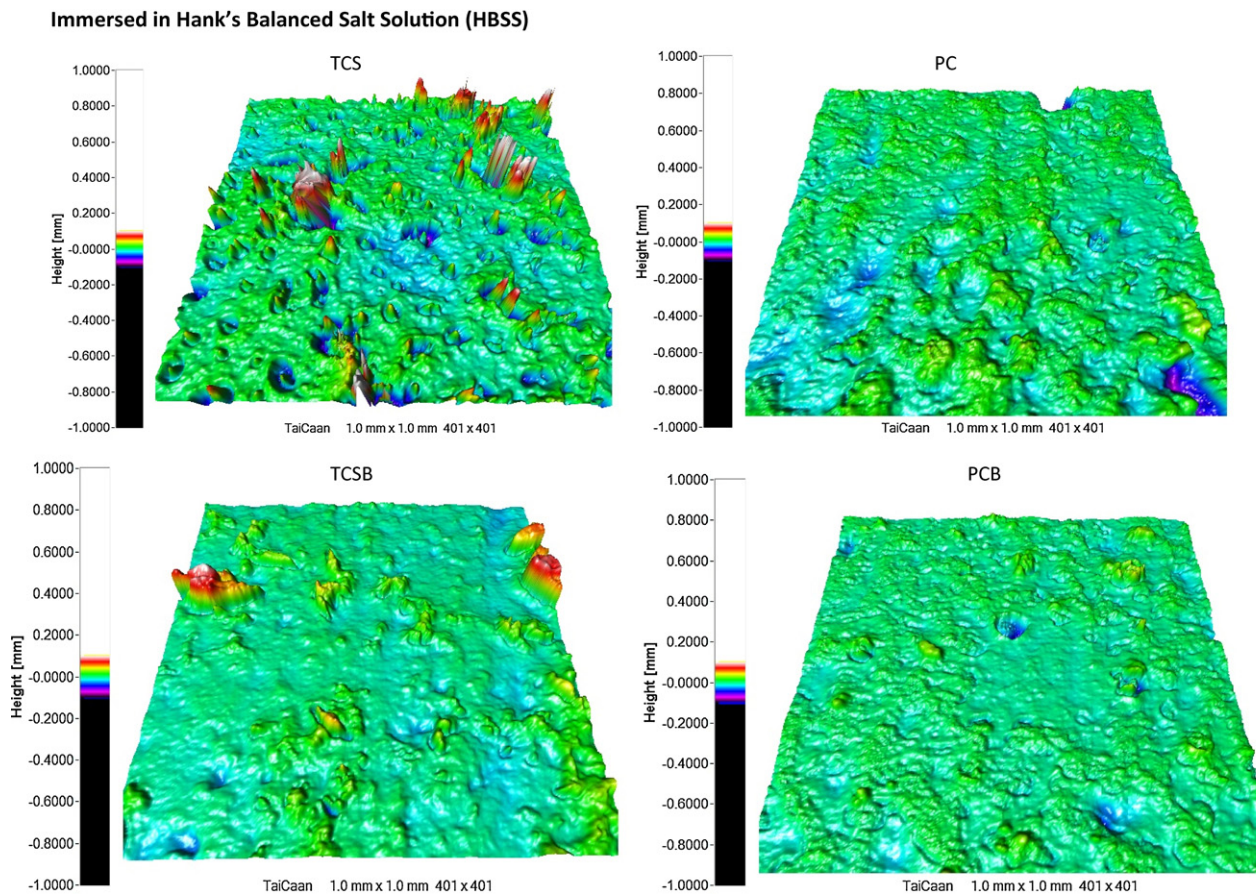**Immersed in Hank's Balanced Salt Solution (HBSS)**

Fig. 5 – (continued)

The characteristic peaks of tricalcium silicate were evident in all the traces except the surface scans of the specimens immersed in distilled water or HBSS, and this confirms that tricalcium silicate is one of the phases present in Portland cement. In the 28-day solid XRD specimens only the calcium carbonate peaks at 13.5° 2θ and 21.5° 2θ were observed. This was caused by the deposition of a layer of surface precipitates that was of sufficient thickness to block out the peaks expected of the underlying cement and thus only the peaks of the surface layer (calcium carbonate) were detected. Conversely in the case of the powdered specimens, both the surface layers and the core of the cement were mixed thoroughly when the discs were crushed into powder, and thus all the peaks were present after 28 days. The peaks were identified using the ICCD database. Unfortunately, the major peaks of calcium carbonate and calcium phosphate coincide at the same angle, around 13.5° 2θ and thus it is impossible to differentiate between them using XRD. However calcium carbonate was able to be confirmed from its secondary peak at 21.5° 2θ which was evident in the diffractograms.

No significant agglomeration of bismuth oxide was observed, indicating that segregation did not occur. Furthermore, no significant difference was observed between the surface morphologies of the tricalcium silicate and bismuth-replaced tricalcium silicate, which indicates that they should be expected to possess similar in vivo properties regarding interaction with the cells in the body. The identification of the phases present was facilitated by EDX scans.

Tricalcium silicate cement was more bioactive than Portland cement as demonstrated by various characterization techniques. The bioactivity was monitored by measuring the production of calcium hydroxide and the formation of calcium phosphate when in contact with simulated body fluids.

Acknowledgments

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