

Characterization of un-hydrated and hydrated BioAggregate™ and MTA Angelus™

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Abstract

Objectives BioAggregate™ is a novel material introduced for use as a root-end filling material. It is tricalcium silicate-based, free of aluminium and uses tantalum oxide as radiopacifier. BioAggregate contains additives to enhance the material performance. The purpose of this research was to characterize the un-hydrated and hydrated forms of BioAggregate using a combination of techniques, verify whether the additives if present affect the properties of the set material and compare these properties to those of MTA Angelus™.

Methods Un-hydrated and hydrated BioAggregate and MTA Angelus were assessed. Un-hydrated cement was tested for chemical composition, specific surface area, mineralogy and kinetics of hydration. The set material was investigated for mineralogy, microstructure and bioactivity. Scanning electron microscopy, X-ray energy dispersive spectroscopic analysis, X-ray fluorescence spectroscopy, X-ray diffraction and isothermal calorimetry were employed. The specific surface area was investigated using a gas adsorption method with nitrogen as the probe.

Results BioAggregate was composed of tricalcium silicate, tantalum oxide, calcium phosphate and silicon dioxide and was free of aluminium. On hydration, the tricalcium silicate

produced calcium silicate hydrate and calcium hydroxide. The former was deposited around the cement grains, while the latter reacted with the silicon dioxide to form additional calcium silicate hydrate. This resulted in reduction of calcium hydroxide in the aged cement. MTA Angelus reacted in a similar fashion; however, since it contained no additives, the calcium hydroxide was still present in the aged cement. Bioactivity was demonstrated by deposition of hydroxyapatite. BioAggregate exhibited a high specific surface area. Nevertheless, the reactivity determined by isothermal calorimetry appeared to be slow compared to MTA Angelus. The tantalum oxide as opposed to bismuth oxide was inert, and tantalum was not leached in solution. BioAggregate exhibited high calcium ion release early, which was maintained over the 28-day period as opposed to MTA Angelus, which demonstrated low early calcium ion release which increased as the material aged.

Conclusions The mineralogical composition of BioAggregate was different to MTA Angelus. As opposed to MTA Angelus, BioAggregate did not contain aluminium and contained additives such as calcium phosphate and silicon dioxide. As a consequence, BioAggregate reacted more slowly and formation of calcium hydroxide and leaching of calcium ions in solution were not evident as the material aged. The additives in BioAggregate modify the kinetics and the end products of hydration.

Clinical significance Although newer generation tricalcium silicate-based materials contain similar constituents to MTA, they do not undergo the same setting reactions, and thus, their clinical performance will not be comparable to that of MTA.

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Introduction

BioAggregate™ (Verio Dental Co. Ltd. Vancouver, Canada) is a tricalcium silicate-based material introduced for use as root-end filling material, for perforation repair, vital pulp therapy and apexification procedures. BioAggregate is composed of tricalcium silicate, hydroxyapatite, amorphous silicon dioxide and tantalum oxide. The tricalcium silicate is the main component phase, and tantalum oxide is added as a radiopacifier. BioAggregate does not contain an aluminate phase or calcium sulphate [1]. The chemical composition has been verified by X-ray diffraction analyses of both the set and un-hydrated forms of the material [2]. BioAggregate exhibited a radiopacity higher than the recommended 3-mm thickness of aluminium and compressive strength and microhardness values similar to that of Intermediate Restorative Material. In addition, it displayed low washout but very extended setting time and high fluid uptake [3].

Hydration of BioAggregate resulted in the formation of calcium hydroxide. Calcium carbonate was also observed in the set forms of the material but not in the un-hydrated powder [2]. This was verified by a later study where hydration by-products were shown to be calcium silicate hydrate and calcium hydroxide, the latter being leached in solution. The leaching of calcium increased to day 14 after which a plateau was reached. The leachate exhibited a very alkaline pH at all time intervals tested namely 1–28 days. BioAggregate exhibited hydrating cement particles with associated reaction by-product around the cement particle and in the cement matrix [4]. Exposure of BioAggregate to physiological solution resulted in precipitation of apatite crystalline structures that increased over time. This suggests that the material is bioactive [5]. BioAggregate has also been shown to be biocompatible and enhanced the differentiation of human fibroblasts [6, 7], osteoblasts [8] and mesenchymal cells [9]. In addition, it exhibited antifungal [10] and antibacterial [11] properties.

Although a number of studies have been performed to assess the clinical performance and bioactivity and biocompatibility of the hydrated material, the research on characterization of the un-hydrated material is still limited. The constituents of BioAggregate are similar to those of mineral trioxide aggregate (MTA) with regard to the presence of a cementitious phase and a radiopacifier. In BioAggregate, tricalcium silicate and tantalum oxide are used instead of Portland cement and bismuth oxide present in MTA. This was presumably done to avoid the aluminium phase and the trace elements shown to be present in MTA [12–15]. Bismuth oxide leaches in the long-term [16], and it has been implicated in dental discoloration [17]. Furthermore, BioAggregate contains additives to the powder, which are claimed by the manufacturer to effect the cement hydration.

The purpose of this research was to verify the effect of additives on the hydration mechanism of BioAggregate by

characterization of the un-hydrated and hydrated forms using a combination of techniques and compare these properties to MTA Angelus™.

Materials and methods

BioAggregate (Verio Dental Co. Ltd. Vancouver, Canada) and MTA Angelus (Angelus Soluções Odontológicas, Londrina PR, Brazil) mixed with water at a water to powder ratio of 0.35 were investigated.

Chemical composition and mineralogy

The quantitative chemical composition of the un-hydrated cement was performed by X-ray fluorescence (XRF; Bruker S4 Explorer, Germany) on pellets made with lithium tetraborate. Before performing XRF analysis, the samples were heated to 1,000 °C to check if the sample contained carbonated or hydrate minerals in order to correct the XRF analysis accordingly.

Phase analysis was carried out on the un-hydrated powder and set cements. For the un-hydrated cements, a diffractometer (Rigaku, Tokyo, Japan) using Cu K α radiation at 40 mA and 45 kV was used. Samples were presented in powder form, and the detector was rotated between 15° and 45°, a sampling width of 0.05° and 2°/min scan speed. Phase identification was accomplished by use of search-match software utilizing International Centre for Diffraction Data (ICDD, Newtown Square, PA, USA) database.

The set cement paste was stored in Hank's balanced salt solution (HBSS) for 1 and 28 days at 37 °C in an incubator prior to testing using X-ray diffraction (XRD). The composition of HBSS was 0.4 M KCl, 0.06 M KH₂PO₄ anhydrous, 0.35 M NaHCO₃, 8.0 M NaCl, 0.05 M Na₂HPO₄ anhydrous and 1.0 M D-glucose. The cement pastes were crushed using a mortar and pestle prior to testing.

Microscopy and elemental analysis

The un-hydrated cement was mounted in resin (Araldite, Huntsman Advanced Materials, Duxford, Cambridge, UK) and polished using a series of diamond discs and polishing cloths of reducing abrasiveness. The resin block was carbon coated and viewed in back-scatter mode under the scanning electron microscope (SEM; Hitachi S4300 SE/N, Bunkyo-ku, Tokyo, Japan). Elemental analysis by X-ray energy dispersive spectroscopy (EDS) was also performed.

Microscopy and EDS analysis were also performed on the cement paste. The samples were dried in acetone ether rinse before being oven dried at 40 °C for 1 week. The reaction rims around the remaining un-hydrated minerals and the radiopacifier phase were observed. Hydration characteristics

of the cement were assessed by plotting the calcium to silicon ratios of the cement particle and the reaction rims. The inclusion of the radiopacifier phase in the cement hydration process was assessed by plotting the radiopacifier to calcium ratio plots of the radiopacifier phase and surrounding cement matrix. Surface topography of the hydrated cements immersed in HBSS at 37 ± 1 °C after 1 day was also performed in order to investigate the bioactivity potential of the materials.

Assessment of material fineness

The specific surface area of the un-hydrated BioAggregate and MTA Angelus was determined by the Brunauer-Emmett-Teller (BET) gas adsorption method using extended pressure sorption analyzer (Micromeritics ASAP 2010, Micromeritics Instrument Corporation, Norcross, GA, USA). Nitrogen gas was used at different pressures to determine the physical adsorption of gas molecules on a solid surface.

Calorimetry

The kinetics of hydration of BioAggregate and MTA Angelus was followed by isothermal calorimetry performed on cement paste at 37 °C. The heat flux released by the chemical reaction was monitored over time and enabled the estimation of the beginning of setting and the rate of increase of the mechanical performance of the cement paste. The calorimeter was composed of fluxmeters that allowed the calorimeter to equilibrate in less than 2 min after the cells were placed in it. Thus, it was possible to record the heat flux released until the hydration beginning even if the mixing of the cement paste was performed outside the calorimeter.

Leachate analysis

Assessment of leaching was performed using inductively coupled plasma (ICP) on leachate recovered from specimens measuring 15 mm in diameter by 2 mm high immersed in 10 mL HBSS for 1, 14 and 28 days. The leachate was assessed for calcium, silicon, tantalum, aluminium, phosphorus and bismuth. Ion release was calculated taking into consideration the sample size and volume of solution used. A blank HBSS solution was also analyzed in order to calculate the ion content in the soaking solution.

Results

Chemical composition and mineralogy

The results of the quantitative analysis of the un-hydrated materials by XRF are shown in Table 1. Both materials were

Table 1 Elemental composition of the un-hydrated BioAggregate and MTA Angelus using X-ray fluorescence

Elements identified	BioAggregate (%)	MTA Angelus (%)
Oxygen	37.4	34.2
Calcium	38.5	43.4
Silicon	11.5	7.6
Aluminium	–	1.5
Magnesium	0.1	0.1
Phosphorus	1.9	–
Tantalum	10.6	–
Bismuth	–	12.8

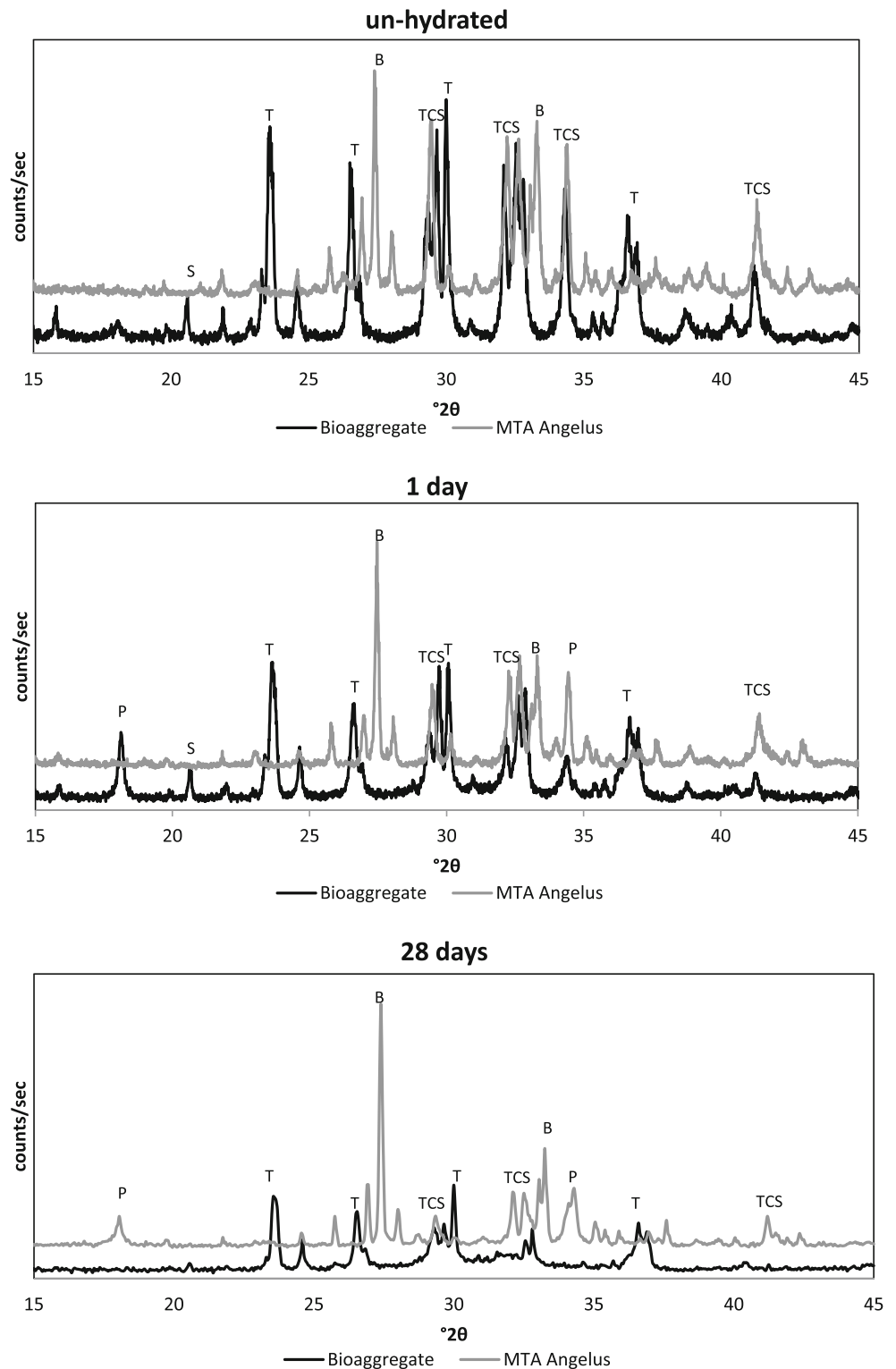
composed primarily of oxygen, silicon and calcium with traces of magnesium. MTA Angelus exhibited the presence of aluminium, while BioAggregate had phosphorus. Tantalum was present in 10 % proportions in BioAggregate while bismuth in 12 % in MTA Angelus.

The diffractograms of the un-reacted and the set materials stored in HBSS for 1 and 28 days are shown in Fig. 1. Both BioAggregate and MTA Angelus exhibited peaks for tricalcium silicate (ICDD 031-0301). Triclinic tantalum oxide (ICDD 021-1198) and silicon dioxide (ICDD 082-1576) were present in BioAggregate, while bismuth oxide (ICDD 071-0465) was present in MTA Angelus. No crystalline phosphorus-containing phases were detected in BioAggregate thus inferring that these phases were amorphous. The main diffraction peak for silicon dioxide in BioAggregate was not intense and broad indicating that silicon dioxide was partly amorphous. It was evident that the tricalcium silicate reacted early in the hydration reaction of BioAggregate when compared to MTA Angelus as after 1 day, the tricalcium silicate peaks were less distinct when compared to the un-hydrated cement. Furthermore, at 28 days while the tricalcium silicate peaks were completely obliterated for BioAggregate, such peaks were still evident for MTA Angelus. The peak intensity of tantalum oxide was generally stable, but a reduction in the peak intensity of bismuth oxide was evident. Portlandite peak was present in the cement stored for 1 day. This was indicative of the presence of calcium hydroxide in the cement paste resulting from tricalcium silicate hydration for BioAggregate but not for MTA Angelus, while the opposite happened at 28 days of hydration.

The scanning electron micrographs of the un-hydrated powders and the EDS analysis of the constituent phases are shown in Fig. 2a. The un-hydrated cements were composed of different particles indicating that the cements were prepared by mixing different materials. The particles and especially the calcium silicate particles were quite coarse as mostly were larger than 20 µm.

The EDS analysis exhibited peaks for calcium and silicon, on specific particles whereas oxygen was always present. In

Fig. 1 X-ray diffraction patterns of un-hydrated and hydrated Bioaggregate and MTA Angelus after immersion in Hank's balanced salt solution after 1 and 28 days (*B* bismuth oxide, *P* portlandite/calcium hydroxide, *S* silicon dioxide, *T* tantalum, *TCS* tricalcium silicate)



addition, BioAggregate exhibited large shiny particles composed of tantalum, while the shiny particles in MTA Angelus were elongated and composed of bismuth. Porous particles that exhibited peaks for calcium and silicon were present. Furthermore, in BioAggregate, elongated particles were

interspersed with the porous particles and exhibited large peaks for calcium and phosphorus. Smaller particles contained mostly silicon.

The scanning electron micrographs of the hydrated cements are shown in Fig. 2b. The tricalcium silicate hydrated

producing reaction rims around the tricalcium silicate grains. The calcium to silicon ratio reduced at the reaction rim indicating the formation of calcium silicate hydrate that is poorly crystallized and thus not detected by XRD. The tantalum to calcium ratio reduced drastically in the cement matrix for BioAggregate, while bismuth lingered in the cement matrix at a longer distance from the bismuth particle in MTA Angelus.

The surface topography of BioAggregate and MTA Angelus soaked in HBSS is shown in Fig. 2c. Both materials exhibited a honeycomb appearance signifying the presence of calcium silicate hydrate (C-S-H). Hydroxyapatite crystals were also evident in all specimens and their presence verified by EDS analysis (Fig. 2c).

Assessment of material fineness

The specific surface area of BioAggregate™ was 9.32 m²/g, which is large taking into account the coarse size of the particles. Thus, the internal porosity mostly of the calcium silicate-containing particle is expected to be largely responsible of the measured specific surface area. MTA Angelus™ had a specific surface area of 1.03 m²/g.

Calorimetry

The results for the calorimetry of both BioAggregate and MTA Angelus are shown in Fig. 3. The heat flux generated for BioAggregate during hydration in water at 37 °C presented an initial endothermic peak which is due to the wetting of the surface. This endothermic effect was not observed for MTA Angelus because it was overcome by the strong endothermic effect induced by the dissolution of tricalcium silicate and tricalcium aluminate and free lime. The main exothermic phase for BioAggregate started at 8 h and ended at 19 h. Its maximum at 14 h had a very low intensity indicating a low reaction rate. Both the presence of an initial endothermic peak and a small and delayed exothermic peak indicate a very low initial reactivity of BioAggregate when compared to MTA Angelus.

Leachate analysis

The results for leachate analysis are shown in Table 2. BioAggregate leached calcium ions in solution reaching a peak at 1 day and maintaining the level by 28 days. On the other hand, MTA Angelus exhibited a low level of calcium ion

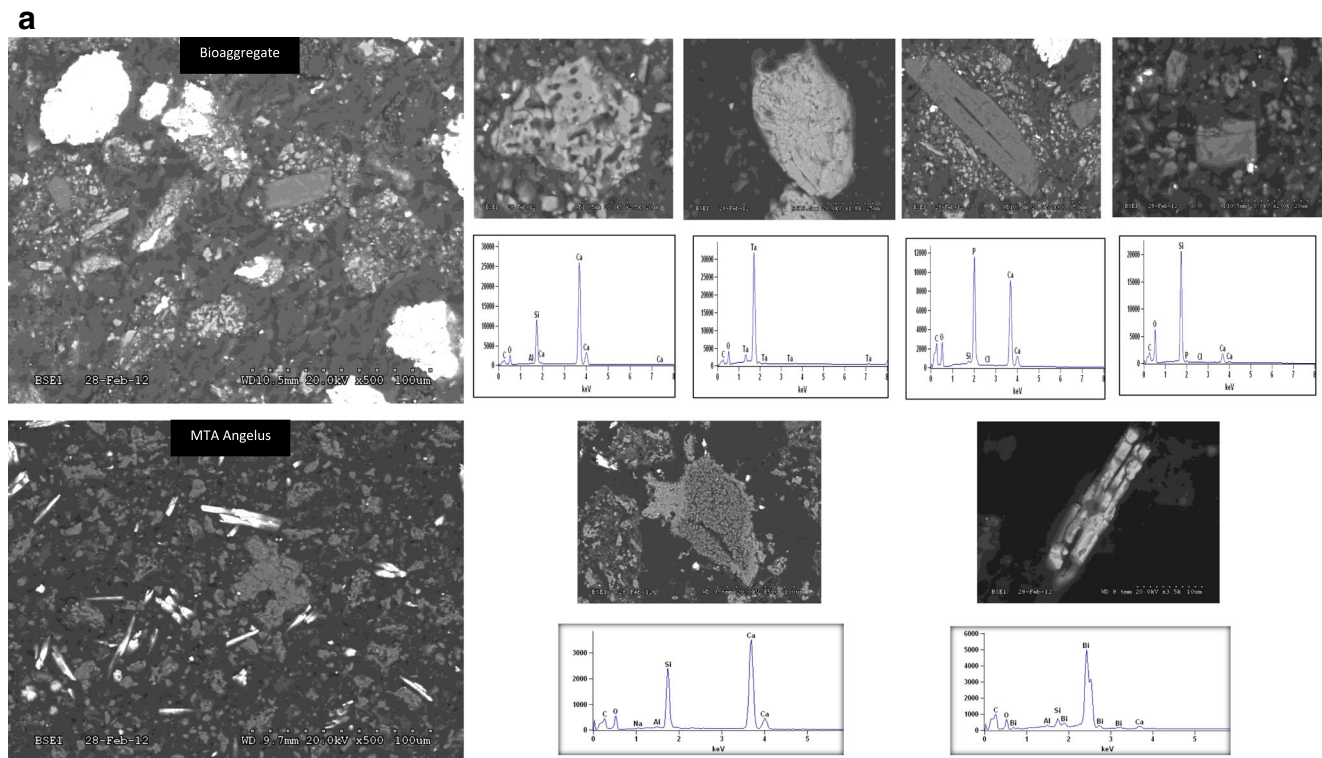


Fig. 2 a Backscatter scanning electron microscopy and energy dispersive spectroscopic analysis of un-hydrated Bioaggregate and MTA Angelus demonstrating the different constituent phases. b Backscatter scanning electron microscopy of hydrated Bioaggregate and MTA Angelus characterizing the different component phases and calcium to silicon and

tantalum to silicon ratio plots. c Scanning electron microscopy of material surface exposed to Hank’s balanced salt solution for 28 days showing globular crystalline precipitates, energy dispersive spectroscopic analysis of crystalline precipitates

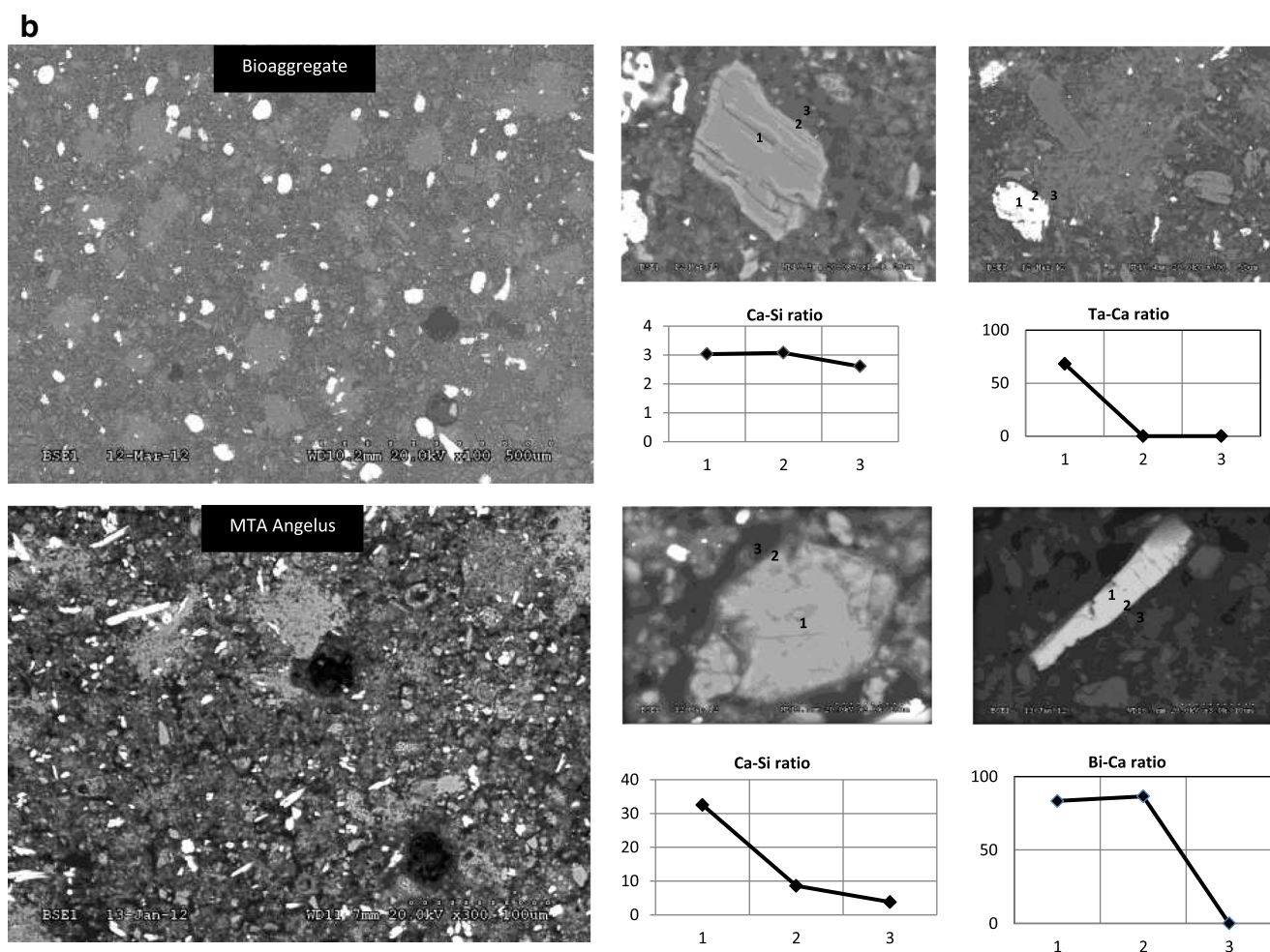


Fig. 2 continued.

leaching in the early stages of hydration, which increased over the 28-day period. BioAggregate released minimum amounts of silicon and phosphorus in solution, while MTA Angelus leached some aluminium. No tantalum was present in the leachate for both materials, but bismuth was leached from MTA Angelus in increasing amounts.

Discussion

Both BioAggregate and MTA Angelus powders were composed of tricalcium silicate and included a radiopacifier phase, tantalum oxide and bismuth oxide, respectively. This was verified by the XRD analysis. BioAggregate also included amorphous hydroxyapatite and mostly amorphous silicon dioxide to the powder as suggested by the manufacturer [1]. The silicon dioxide was detected on XRD with a broad peak and was evident on the electron micrographs and elemental analysis. The calcium phosphate was only identified in the

scanning electron microscopy and EDS traces. The tantalum oxide, calcium phosphate and silicon dioxide were also identified by other researchers investigating the composition of BioAggregate [2]. The material was free of aluminium. This was verified by XRF and EDS analyses. Water was used as the mixing liquid in the current study as bioliquid A, the liquid supplied with BioAggregate, was shown to be water in a previous study [18].

The material fineness was calculated using the BET gas adsorption method. The BET method is widely used in surface science for the calculation of surface areas of solids by physical adsorption of gas molecules. By application of the BET theory, it is even possible to determine the inner surface of hardened cement paste. BioAggregate exhibited a specific surface area of approximately $9 \text{ m}^2/\text{g}$ as opposed to the much smaller surface area exhibited by MTA Angelus. This value was high as most of the particle size observed by SEM was greater than $20 \mu\text{m}$. The reported value is also greater than the values of specific surface areas reported for MTA PlusTM and ProRoot MTA[®] [19] and other related materials [20]. The

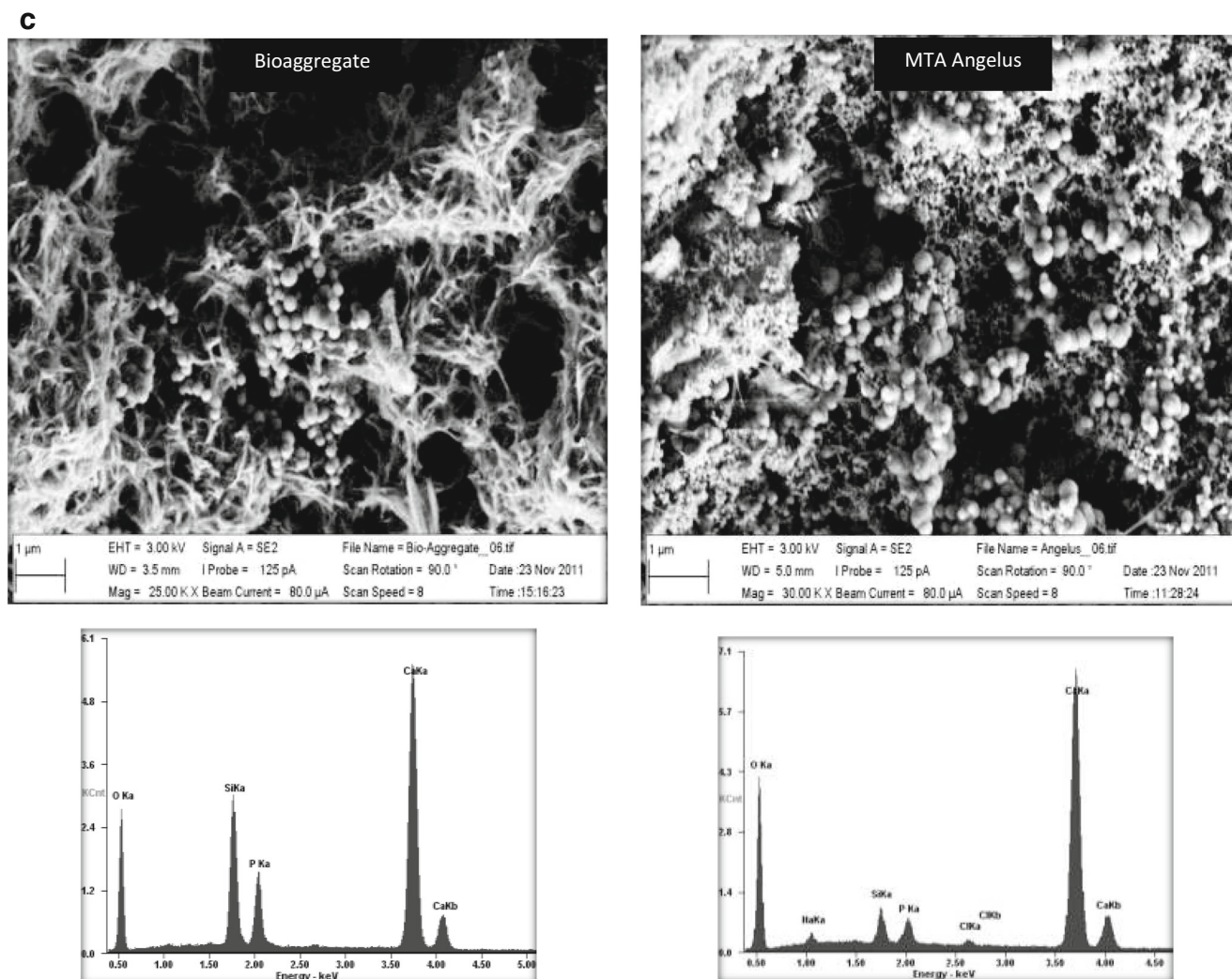


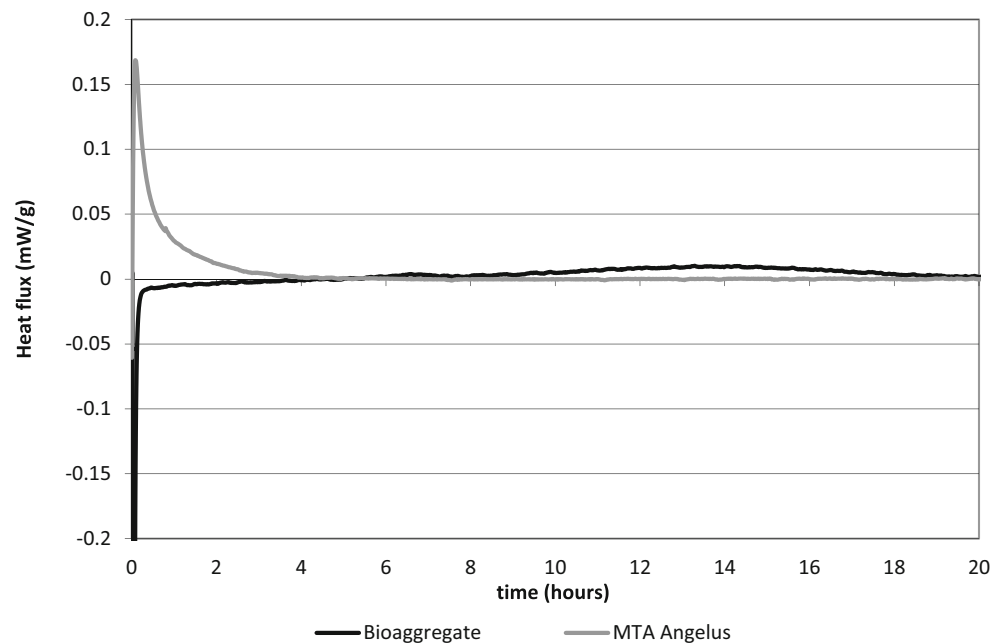
Fig. 2 continued.

high specific surface area is certainly caused by the internal porosity of the calcium silicate particles, which was evidenced by the scanning electron microscopy of the un-hydrated BioAggregate. The high specific area surface area and particle internal porosity result in the need of more water to obtain an adequate mix when water are added to the powder. High water to cement ratios result in increased material porosity and a reduction in material strength. The high leaching rate of BioAggregate demonstrated in the current study verifies the high material porosity of the set cement. This is clinically undesirable as the increased porosity may attract more bacterial adhesion to the material surface. Although BioAggregate is used as a root-end filling material and thus is placed in non-stress-bearing areas, poor physical characteristics will lead to material deterioration with possible dislodgement and loss caused by high solubility. BioAggregate exhibited poorer physical characteristics than Biodentine and IRM in a recent study [3]. Furthermore, the coarse calcium silicate grains led

to a slow rate of hydration of BioAggregate as demonstrated by isothermal calorimetry.

The amorphous silicon dioxide reacted with the calcium hydroxide in the hydrated cement to form additional calcium silicate hydrate. The elimination of calcium hydroxide was verified in the current study by the absence of the portlandite peak at 28 days. Calcium hydroxide content can be reduced by addition of latent hydraulic binders which are usually very fine particles with high specific surface area, X-ray amorphous and a high percentage of total aluminium and silicon (FAS) content. These materials react with the calcium hydroxide and produced more cementitious phases thus enhancing the mechanical properties of the cement and reducing its permeability. Such latent hydraulic binders are used routinely in the construction industry. Amorphous silicon dioxide is a very good candidate as a latent hydraulic binder. Assessment of the physical properties of BioAggregate exhibited low levels of both compressive strength and micro-hardness when

Fig. 3 Results for heat flux generated in Bioaggregate and MTA Angelus on hydration with time



compared to other root-end filling materials notably Biodentine® and IRM® [3]. Thus, the latent hydraulic binder effect although evident chemically due to the absence of portlandite peak at 28 days was not evident by improvement in the physical properties. A high leaching rate that is certainly correlated to a high porosity of the set material was evident from the leaching experiments. Improvement in the physical properties of BioAggregate can be achieved by the addition of a water-soluble polymer as is employed in Biodentine [20]. This will result in a decrease of the solid to liquid ratio when preparing the BioAggregate paste. MTA Angelus did not have any additives as evidences by the presence of the portlandite peak at 28 days. The absence of calcium hydroxide at 28 days of hydration will have a negative influence on the bioactivity of BioAggregate. Calcium silicate-based materials exhibit bioactivity as a function of the reaction of calcium hydroxide produced as a by-product of hydration, which

interacts with tissue fluids to form hydroxyapatite on the material surface. This phenomenon was initially reported for MTA [21–23] but has been demonstrated with most of the tricalcium silicate-based materials [5, 20].

Both BioAggregate and MTA Angelus demonstrated bioactivity, and hydroxyapatite deposits were evident on the set material. This was verified micro-structurally by scanning electron microscopy. This is in accordance to a previous study investigating the bioactivity of BioAggregate [5]. Hydroxyapatite has been proposed as an addition to root-end filling materials in order to enhance their ability to form a biochemical bond to the bone [24]. XRD analysis demonstrated low levels of calcium hydroxide as the material aged. The ICP analysis indicted very high early release of calcium, which stabilized by 28 days of hydration. This is in accordance to the XRD assessment showing no formation of calcium hydroxide in the aged material. The lack of calcium hydroxide as the material ages may cause a reduction in material bioactivity

Table 2 Leaching of elements in solution identified by inductively coupled plasma

Elements identified (mg/g)	BioAggregate 1 day	MTA Angelus	BioAggregate 14 days	MTA Angelus	BioAggregate 28 days	MTA Angelus
Ca	14,996	403.58	11,188	5,874	14,735	10,096
Si	65.07	0	41.30	0	50.60	0
P	6.10	0	1.29	0	1.81	0
Al	0	4.60	0	2.23	0	2.27
Ta	0	0	0	0	0	0
Bi	0	7.15	0	21.93	0	19.32

and biocompatibility in the long-term. The effects of the latent hydraulic binder on BioAggregate with regard to the biological properties need to be further investigated.

The tantalum oxide in BioAggregate seemed to act as a filler in the cement matrix as there was no evidence of the presence of tantalum in the reaction rim around the hydrating cement particle as opposed to the presence of bismuth in the reaction rim of the hydrating MTA Angelus. Furthermore, no leaching of tantalum was observed in the ICP analysis as opposed to the leaching of bismuth from MTA. The evidence of leaching of bismuth from MTA is in accordance to previous studies performed on ProRoot MTA [16]. The absence of bismuth oxide reduces the risk of tooth discoloration [17] although with root-end filling materials, this is not a major clinical problem.

MTA Angelus leached aluminium as verified by ICP analysis. MTA Angelus includes Portland cement as the main cementitious phase as opposed to the tricalcium silicate in BioAggregate. The latter lacks the aluminate phase and thus exhibits no leaching of aluminium in solution. Release of aluminium ions in solution is undesirable as aluminium has been linked with Alzheimer's disease [25]. The use of pure tricalcium silicate cement as opposed to Portland cement which contains calcium aluminate avoids the leaching of aluminium in solution thus in contact with host tissues. Furthermore, the use of pure tricalcium silicate cement also avoids the presence of trace elements that are usually found in Portland cement and MTA [12–15]. No leachable trace elements were present in BioAggregate as demonstrated in a recent study [18].

Conclusions

The mineralogical composition of BioAggregate was different to that of MTA Angelus. As opposed to MTA Angelus, BioAggregate did not contain aluminium and contained additives such as calcium phosphate and silicon dioxide that were partly amorphous. As a consequence, BioAggregate reacted more slowly, and formation of calcium hydroxide and leaching of calcium ions in solution were not evident as the material aged. Further investigation on the improvement of BioAggregate properties is warranted.

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Conflict of interest The authors declare that they have no conflict of interest.

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