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Microstructure and chemical analysis of blood-contaminated mineral trioxide aggregate

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Abstract

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Aim To test the hypothesis that blood contamination has a detrimental effect on the chemical properties of Mineral trioxide aggregate (MTA).

Methodology The effects of whole, fresh human blood on the microstructure and elemental chemistry of MTA were evaluated using scanning electron microscopy and energy-dispersive X-ray analysis, respectively. The phase compositions of contaminated and uncontaminated MTA were also analysed using X-ray diffraction analysis.

Results The hydration state of specimens partially mixed with blood were more complete than those mixed entirely with blood and less than those entirely mixed with water. Acicular crystals, characteristic of ettringite, were abundant in specimens mixed entirely with water and absent from specimens mixed partially or entirely with blood. Calcium hydroxide crystals were absent in specimens contaminated entirely with blood and the unhydrated MTA powder, but present in the other groups.

Conclusion Mixing MTA with blood resulted in the lack of formation of the crystalline calcium hydroxide in the early stage of the hydration process.

Keywords: energy-dispersive X-ray, Mineral Trioxide Aggregate, mineral trioxide aggregate, scanning electron microscopy, X-ray diffraction analysis.

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Introduction

Mineral trioxide aggregate (MTA) is a hydraulic cement which, in contact with water, forms a nonresorbable and dimensionally stable material (De-Deus *et al.* 2008). MTA is a derivative of Portland cement (Estrela *et al.* 2000, Camilleri *et al.* 2005) that is biocompatible (Torabinejad *et al.* 1995a), capable of close adaptation to dentine (Shokouhinejad *et al.* 2010) and has antibacterial activity (Torabinejad *et al.* 1995b). MTA has also the ability to induce hard tissue formation when it comes into contact with connective tissue (Shabahang *et al.* 1999) and is considered as the material of choice in vital pulp therapies (Nakashima & Akamine 2005), for the repair of accidental and/or pathological root perforations (Pitt Ford *et al.* 1995), as an apical root canal plug in immature teeth with necrotic pulp tissue (Witherspoon & Ham 2001) and as a root-end filling material in endodontic surgery (Torabinejad *et al.* 1993). In all of these clinical situations, MTA comes into contact with blood (Nekoofar *et al.* 2010c).

Torabinejad *et al.* (1994) reported that blood contamination did not have a significant effect on the sealing properties of MTA, which was subsequently confirmed by Montellano *et al.* (2006). In contrast, Vanderweele *et al.* (2006) reported that MTA had significantly less resistance to displacement from blood-contaminated perforation sites and advised removal of blood before its placement. Nekoofar *et al.* (2010b,c) demonstrated a reduction in the surface microhardness and compressive strength of blood-contaminated MTA and linked these reductions with the impeded formation of acicular crystalline structures. However, the

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resultant materials were not subjected to elemental or phase composition analysis.

In this study, the effects of whole, fresh human (WFH) blood contamination on the surface microstructure and elemental chemistry of MTA, using scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) analysis, respectively, were analysed. The phase compositions of contaminated and uncontaminated MTA were also determined using X-ray diffraction analysis (XRD).

Materials and methods

The material investigated was tooth-coloured ProRoot-MTA (LOT number 09001920; Dentsply Tulsa Dental, Johnson City, TN, USA). WFH blood was collected from a healthy consented volunteer in accordance with the Declaration of Helsinki ethical principles (2000).

Sample preparation

Mineral trioxide aggregate slurries were prepared according to the following three groups.

Group 1 MTA mixed with distilled water

Group 2 MTA mixed with WFH blood diluted with distilled water (50% v/v)

Group 3 MTA mixed with WFH blood

Mixing of MTA was standardised by triturating 1 g of MTA powder and 0.33 g of liquid in a plastic mixing capsule containing a plastic pestle (Nekoofar *et al.* 2010a), at 4500 revolutions min^{-1} for 30 s, using an amalgamator (Promix^{TM}; Dentsply Caulk, York, PA, USA). The resulting MTA slurries were placed with minimal pressure in the circular depression of an XRD sample holder (Panalytical, Almelo, Netherlands). The materials were then subjected to ultrasonic energy using a BUC-1 Spartan tip (Obtura Spartan, Fenton, MO, USA) attached to a Suprasson® P5 Booster (Satelec, Cedex, France), to standardise the placement technique. The ultrasonic tip was moved throughout the MTA slurry without touching either the wall or floor of the sample holder, whilst being activated for 30 s at power scale 5 (Nekoofar *et al.* 2010a). Specimens were then incubated at 37 °C in fully saturated humidity for 4 days.

SEM and EDX analysis

The surface characteristics of specimens from each group were examined and subjected to elemental analysis. The MTA specimens of each group were broken and mounted on aluminium stubs using adhesive carbon discs and analysed uncoated using a scanning electron microscope (SEM; Carl Zeiss EVO 40, Oberkochen, Germany) fitted with an energy-dispersive X-ray detector (EDX; Oxford Instruments, Oxford, UK). The locations of the images were selected at random from the internal surface of the broken pellet and are considered to be the representative of the material.

XRD analysis

Phase compositions of MTA specimens from each group were determined using an X-ray diffractometer (XRD; Panalytical X'pert pro, Almelo, Netherlands). The specimen surfaces were polished with 1200-grit fine-grain sandpaper (3M; St Paul, MN, USA) to ensure that the surface of the sample was levelled with the holder surface. X-ray diffraction patterns were then recorded using Ni filtered CuK_α radiation (40 Kv and 40 mA). Scans were undertaken in the range 10–80^o 2θ. All patterns were matched using the ICDD database (International Centre for Diffraction Data, Pennsylvania, PA, USA). For comparison, a specimen of unhydrated MTA powder was also subjected to X-ray diffraction analysis as group 4.

Results

SEM & EDX

Scanning electron microscopy analysis of group 1 revealed a homogeneous amorphous layer containing small microchannels (1–30 μm) (Figs 1 and 2) and dispersed with distinct 0- to 10-μm structures (Fig. 1a) shown to have high levels of bismuth by EDX analysis (Fig. 1aii). At higher magnification, clusters of fine acicular (needle-like) crystals were seen (Fig. 1biii). EDX analysis revealed higher sulphur and aluminium concentration in acicular clusters relative to the background matrix (Fig. 1biv), which demonstrated a higher level of calcium, oxygen, silicon, and magnesium in addition to trace quantities of iron.

Scanning electron microscopy analysis of group 2 demonstrated a more highly porous structure (1–30 μm) (Fig. 2b) than that of other groups with a large number of air voids (30–50 μm diameter). SEM analysis of group 3 demonstrated incomplete crystal formations with less angular structures. Large unhydrated particles were seen embedded in the partially fused matrix of group 3. The acicular crystals abundant in group 1 were absent from both other groups.

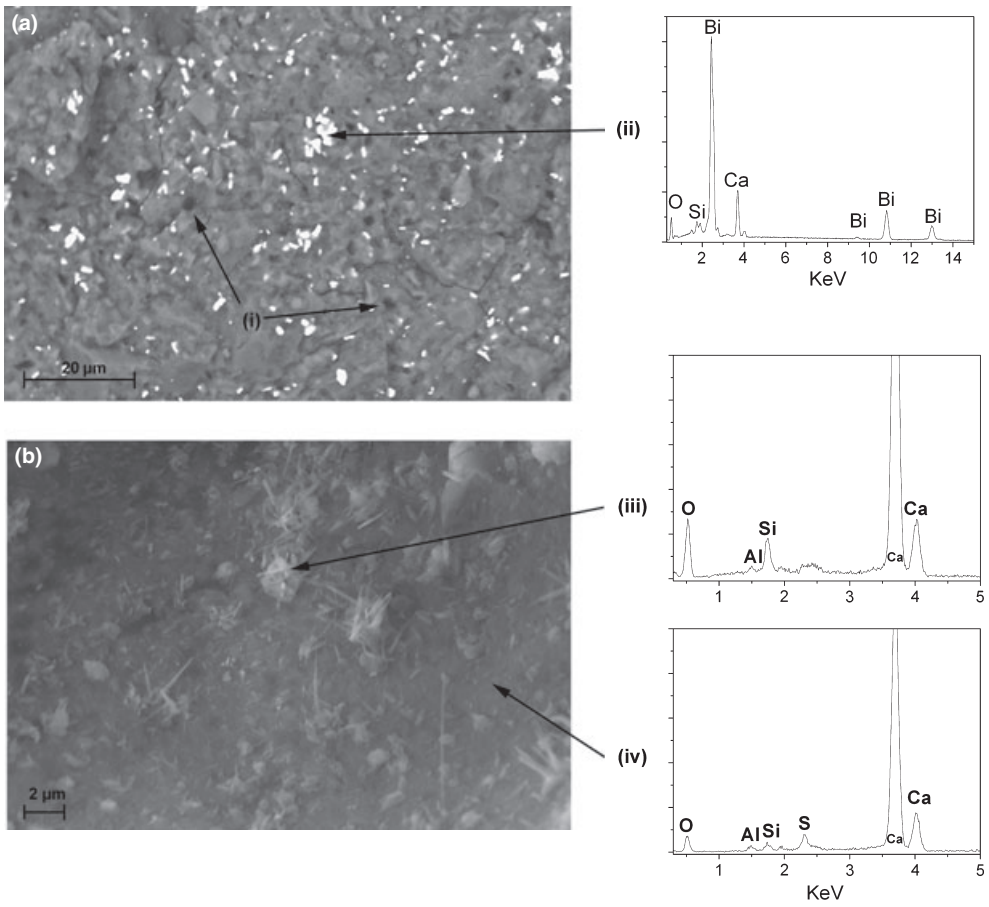

Figure 1 Scanning electron microscopy image of uncontaminated hydrated WMTA (a) showing small microchannels (i) amongst distinct structures shown to have high levels of bismuth oxide by energy-dispersive X-ray (EDX) analysis (ii). Higher magnification SEM of uncontaminated hydrated WMTA (b) demonstrating the presence of acicular crystals (iii) on an amorphous background matrix (iv). EDX analysis showed the acicular crystals (iii) to have a higher level of sulphur and aluminium than the background matrix (iv), characteristic of ettringite (hexacalcium aluminate trisulphate hydrate).

X-ray diffraction analysis

All specimens analysed by XRD comprised bismuth oxide (α-Bi_2O_3, ICDD 00-027-0053) indicated by the main peaks at 27.38, 33.07 and 33.23° 2θ and tricalcium silicate (Ca_3SiO_5, ICDD 00-055-0738) indicated by the peaks at 32.13, 32.56 and 34.30° 2θ (Fig. 3). Levels of tricalcium silicate and bismuth oxide were lower in groups 1 and 2 than in groups 3 and 4. Groups 1 and 2 (Fig. 3-d and 3-c, respectively) showed reflections at 18.10, 28.69 and 34.10° 2θ indicative of calcium hydroxide ($Ca(OH)_2$, ICDD 00-044-1481). The calcium hydroxide phase was absent in group 3 (Fig. 1b) and the unhydrated MTA powder in group 4 (Fig. 3a).

Discussion

Mineral trioxide aggregate is a derivative of Portland cement that consists of tricalcium silicate (alite), dicalcium silicate (belite), tricalcium aluminate (aluminate), tricalcium aluminoferrite (celite), calcium sulphate (gypsum) and bismuth oxide (bismite) (Belio-Reyes *et al.* 2009). MTA can set in contact with water and undergoes a hydration process similar to Portland cement (Camilleri 2007, Lee *et al.* 2007). During the initial stage of the hydration process, Ca^{2+} and OH^- ions are released from tricalcium silicate (C_3S) into the surrounding environment which, at supersaturation levels, forms calcium hydroxide (portlandite) precipitate and amorphous calcium silicate hydrate (CSH) gel

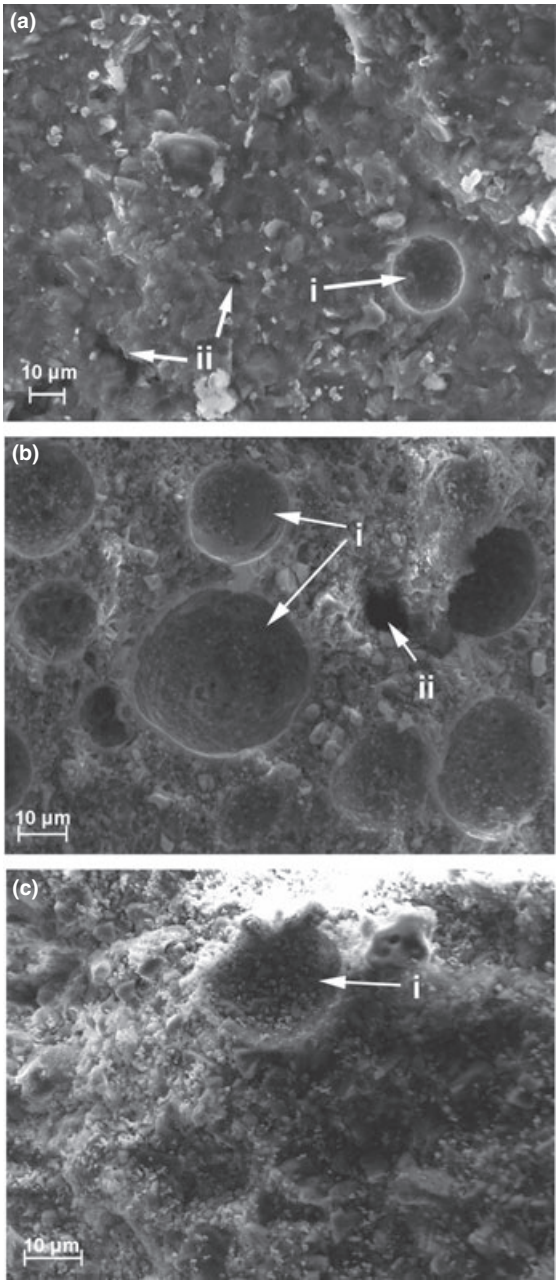

Figure 2 Scanning electron microscopy images of mineral trioxide aggregate specimens mixed entirely with water (a), partially (b) and entirely (c) with whole, fresh human blood, from groups 1, 2 and 3, respectively. Specimens from group 2 demonstrated a more porous (i) structure than other groups. Cross-sections of microchannels (ii) can be seen in groups 1 and 2.

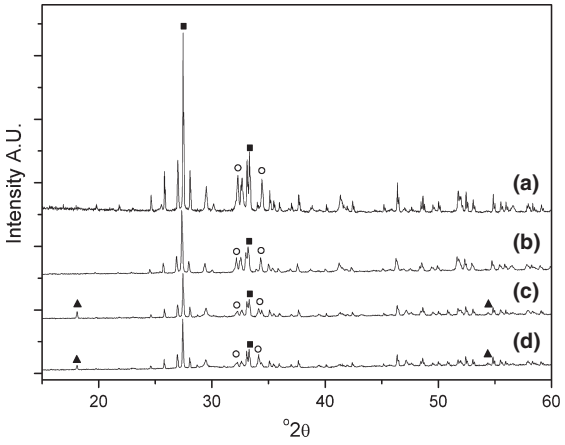

Figure 3 X-ray diffraction patterns showing the main compound present: Unhydrated mineral trioxide aggregate powder group 4 (a), Experimental group 3 (b), Experimental group 2 (c), Experimental group 1(d). Main phases present were highlighted with the following symbols: (■) α-Bi_2O_3, (○) Ca_3SiO_5 and (▲) $Ca(OH)_2$.

(Camilleri 2007). In the presence of sulphate and aluminium ions, crystals of ettringite (hexacalcium aluminate trisulphate hydrate) are also formed (Hewlett 2004, Camilleri 2008, 2010). The sulphate and aluminium ions originate from the dissolution of gypsum and aluminate, respectively (Taylor 1997, Lee *et al.* 2007, Gandolfi *et al.* 2010). The setting and strength of hydraulic cements have been attributed to the formation of CSH and ettringite on nucleation sites of calcium hydroxide (CH) crystals (Taylor 1997, Hewlett 2004, Lee *et al.* 2007). Therefore, the formation of CH in the early stage is crucial for the progression of the hydration process (Groves 1981, Banfill 1986, Hewlett 2004). The antibacterial properties of MTA have been explained by the alkaline environment formed by the release of hydroxide ions (Zhang *et al.* 2009). Additionally, induction of hard tissue has been suggested to be a result of the presence of calcium ions (Shabahang *et al.* 1999).

X-ray diffraction analysis was used in this study to determine the effect of blood contamination on the early stages of the hydration process of MTA. MTA hydration in the presence of solutions containing phosphate ions, such as tissue fluid or blood, resulted in the precipitation of hydroxyapatite (HA) crystals (Sarkar *et al.* 2005), which has also been accredited for the bioactivity of MTA (Reyes-Carmona *et al.* 2009). In the present study, despite MTA being exposed to blood, XRD analysis of MTA specimens did not clearly demonstrate the presence of HA crystals, which may be due to the relatively short exposure period used in this study when compared to longer term exposures

described in other studies (Bozeman *et al.* 2006, Parirokh *et al.* 2009, Reyes-Carmona *et al.* 2009).

In the present study, consistent with Song *et al.* (2006) and Ding *et al.* (2008), to minimize the potential to miss the hydroxyapatite and calcium hydroxide crystals formed on the surface, specimens were not subjected to grinding prior to XRD analysis. Grinding might dilute the surface concentration of HA crystals to lower the XRD detection limit (Coleman *et al.* 2007).

Analysis of specimens mixed with distilled water (group 1) revealed the presence of bismuth oxide, tricalcium silicate and calcium hydroxide. These results are consistent with those found by Camilleri (2008), even though the incubation time and preparation technique were not identical. Accordingly, the proposed hydration mechanism is:

$$2(3CaO.SiO_2) + 6H_2O \rightarrow 3CaO.2SiO_2.3H_2O + 3Ca(OH)_2$$

expressed in cement nomenclature as:

$$2C3S + 6H \rightarrow 3CSH + 3CH$$

Diffractograms of unhydrated MTA powder (group 4) and specimens mixed wholly with WHF blood (group 3) demonstrated a lack of reflections at 18.10, 28.69 and 34.10 $^{o}2\theta$ (▲ symbols in Fig. 3), corresponding to CH, indicating that the formation of crystalline CH had been impeded, which could indicate dissolution of hydroxyl ion. To quantify the formation of various crystals such as C_3S and CH, Rietveld refinement has been suggested (Scrivener *et al.* 2004). Alternatively, to semi-quantify the XRD peaks, an internal standard can be added (Camilleri 2007). However, in the present study, the relative intensity of reflections corresponding to C_3S of group 3 (○ symbols in Fig. 3) was relatively higher than that of groups 1 and 2, suggesting incomplete transition of C_3S to crystalline CH in group 3, and it was not possible to analyse it quantitatively. Moreover, the formation of amorphous CSH should be considered, because in XRD analysis, only crystalline formations are detectable. Additionally, the absence of this amorphous content can be as a result of the short hydration time (Camilleri 2008). Therefore, the evaluation of the specimens after increased incubation time is suggested.

The CH phase was also seen in the diffractogram of specimens mixed with WFH blood diluted with distilled water (group 2). Camilleri (2007) showed that CSH takes up bismuth oxide during the hydration process. Considering the limitation of the nonquantitative analysis, in the present study, the lower relative intensity of bismuth oxide (■ symbols in Fig. 3) shown in group 3, compared with that of the unhydrated specimens, suggests that CSH gel is being formed and taking up bismuth oxide. However, the lower intensity of bismuth oxide in groups 1 and 2, when compared to that of group 3, assumes the impeded hydration of the specimens in the latter group that were mixed entirely with WFH blood.

To better understand the effect of WFH blood contamination on the hydration process of MTA slurries, SEM was also employed. SEM analysis revealed several morphological differences between groups. Clusters of fine acicular crystals were only seen in specimens mixed with distilled water (group 1) and not in specimens partially or wholly mixed with blood (groups 2 and 3, respectively). This characteristic absence of acicular crystals in blood-contaminated MTA has previously been shown by Nekoofar *et al.* (2010b,c). The absence of the acicular crystals was also reported as a result of exposure of MTA specimens to acidic environment (Lee *et al.* 2004). Reduced compressive strength and lack of acicular crystalline structure as a result of acid-etch procedure were also shown by Kayahan *et al.* (2009). Spot analysis of the acicular crystals by EDX indicated that they were rich in sulphur and aluminium as compared to the background matrix (Fig. 2), suggestive of ettringite crystals. Lee *et al.* (2004), Kayahan *et al.* (2009) and Nekoofar *et al.* (2010b,c) associated the lack of acicular crystals with the reductions in compressive strength and surface microhardness of acid and blood-contaminated MTA, respectively.

Despite the similar appearance of the diffractograms of groups 1 and 2, the lack of acicular crystals in specimens that were partially or entirely mixed with blood (groups 2 and 3, respectively) can be explained by the inhibited hydration process owing to the lower water concentration in these groups. Therefore, the SEM and XRD findings demonstrated the hydration state of specimens partially mixed with blood (group 2) to be more complete than that mixed entirely with blood (group 3) and less than that of the fully hydrated specimens (group 1).

Jasiczak & Zielinski (2006) demonstrated the air entrainment effect of red blood cells when mixed with Portland cement. Remadnia *et al.* (2009) revealed that haemoglobin or whole animal blood when used as an admixture to Portland cement resulted in the

increased porosity of the material, which is in accordance with the results of the SEM in the present study (Figs 1 and 2). Specimens in group 3, despite being mixed wholly with WFH blood, had a less porous nature than those of group 2 that were partially mixed with WFH blood. The inhibited hydration process previously explained in group 3 can be the reason for the unexpectedly lower level of porosity relative to other groups. Formation of microchannels and interconnected pore networks is crucial for the full formation of crystalline phases (Hewlett 2004) and the progression of hydration (Fridland & Rosado 2003, Nekoofar *et al.* 2007). The lower porosity of group 3 specimens may be the result of blood protein adhesion to crystal nucleation sites resulting in hydration inhibition (Gandolfi *et al.* 2009). The presence of microchannel cross-sections, evident in SEM images of specimens of groups 1 and 2, provides space for water molecules resulting in an improved hydration process and confirms the XRD and EDX findings. To verify this relationship, quantitative analysis of the hydration phases is suggested.

The specimens partially mixed with blood were created to best simulate the clinical applications of MTA, such as direct pulp capping or repair of root perforations, in which MTA slurries often become partially mixed with blood during and after its placement. In addition, group 3 was included to represent the severe blood contamination that may also be experienced clinically when acute inflammation is present. Coleman *et al.* (2007) investigated the effect of simulated body fluid (SBF), whose ionic composition approximates to that of human plasma (Kokubo & Takadama 2006), on set white Portland cement (WPC) and reported the absence of CH and ettringite as a result of WPC being in contact with SBF for 7 days at 37 °C. They attributed the absence of calcium hydroxide to the dissolution of the hydroxyl ions associated with hydroxyapatite formation (Coleman *et al.* 2007). In the present study, to better replicate the clinical situation, whole fresh human blood was used to contaminate MTA rather than SBF, despite the technical and ethical difficulties involved. Owing to the differing methodologies used, direct comparison of the present findings with those of Coleman *et al.* (2007) is not possible.

Conclusion

The hydration state of specimens partially mixed with blood was more complete than those mixed entirely with blood and less than specimens that hydrated completely with water. Lack of formation of the crystalline calcium hydroxide in the early stage of the hydration process and the absent of acicular crystals, characteristic of ettringite crystals, in blood-contaminated specimens was a common finding.

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