

An evaluation of the effect of blood and human serum on the surface microhardness and surface microstructure of mineral trioxide aggregate

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Abstract

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Aim Short-term and long-term evaluation of the effect of whole human blood or serum contamination on the surface microhardness value and microstructure of white and grey mineral trioxide aggregate (MTA).

Methodology Three groups of 10 samples for each type of MTA were prepared. The first group was mixed with and exposed to fresh whole human blood. The second and third groups were mixed with distilled water and exposed to fresh whole human blood or human serum, respectively. The control group samples were mixed with and exposed to distilled water. During preparation, 1 g of MTA was triturated with 0.33 g of the selected liquid using an amalgamator and placed inside borosilicate cylindrical moulds. The samples were treated with ultrasonic energy. Vickers surface microhardness values were compared after 4 and

180 days. Scanning electron microscopy (SEM) analysis was performed after 4 days.

Results White MTA had a greater microhardness value than grey MTA in all groups. There was a significant difference between the control and the experimental groups ($P < 0.00001$). There was no significant difference between the microhardness values obtained after 4 and 180 days, apart from grey MTA mixed with blood or exposed to serum ($P < 0.00001$). SEM analysis showed the contaminated samples were devoid of acicular crystals that were prominent in the control groups.

Conclusion Blood contamination had a detrimental effect on the surface microhardness of MTA in the short and long term. If blood or serum contamination is unavoidable under clinical conditions, it might be preferable to use white MTA.

Keywords: blood contamination, human serum, mineral trioxide aggregate, scanning electron microscopy, trituration, ultrasonic, Vickers surface microhardness, whole human blood.

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Introduction

Mineral trioxide aggregate (MTA) was originally developed for the repair of root perforations (Lee *et al.* 1993) but is increasingly being used in a wide range

of clinical treatments, such as a root-end filling material after root-end resection (Torabinejad *et al.* 1993), as an apical barrier in immature teeth (Witherspoon & Ham 2001), as a pulp capping agent during vital pulp therapy and pulpotomy in primary and permanent teeth (Bakland 2000, Eidelman *et al.* 2001, Faraco & Holland 2001), as a repair material in tooth resorption (White & Bryant 2002) and as a root canal filling material in the coronal section of

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horizontally fractured teeth (Yildirim & Gencoglu 2009).

An ideal root repair and root-end filling material should not be affected by the contamination of physiological solutions such as blood and/or saliva (Gartner & Dorn 1992, Lemon 1992). However, in view of its various applications, MTA may become contaminated by blood during placement. Torabinejad *et al.* (1994) evaluated the effect of blood contamination on MTA in an *ex vivo* study by comparing the leakage of amalgam, Super EBA, IRM and the preliminary experimental prototype of MTA when applied to root-end cavities that were contaminated by blood immediately after root resection. They reported that there was no significant difference between dye leakage in contaminated and uncontaminated groups and that MTA leaked significantly less than the other materials. In a laboratory bacterial leakage study, Montellano *et al.* (2006) evaluated the effect of blood and/or saliva contamination on bacterial penetration of root-end cavities that were filled by tooth coloured MTA after root-end resection. Saliva-contaminated specimens demonstrated significantly more bacterial penetration than the uncontaminated group. However, contamination or absence of blood had no significant effect on bacterial penetration of root-end cavities that were filled by MTA. Conversely, Vanderweele *et al.* (2006), when evaluating the retention characteristics of MTA in simulated furcation perforations, reported that in the blood contaminated group, MTA had significantly less resistance to displacement compared to the uncontaminated group at 7 days. Therefore, they recommended that blood should be removed before the placement of MTA. In contrast, Arens & Torabinejad (1996) recommended that perforation sites should not be dried before the placement of MTA. In addition, Sluyk *et al.* (1998) reported that the presence of moisture in a perforation site resulted in good adaptation of MTA to the perforation walls. Furthermore, they recommended a moistened matrix be positioned in the perforation defect before placement of MTA for ease of MTA condensation and to prevent over-extrusion of the material. However, Al-Daafas & Al-Nazhan (2007) found the use of an internal matrix beneath MTA, preventing its direct contact with the tissues, produced an adverse healing response and reduced connective tissue attachment and bone formation in the site of the perforation.

The need to condense MTA during placement was evaluated by Nekoofar *et al.* (2007) in an *ex vivo* study. They demonstrated that when high condensation pressures (8.88 MPa) were applied to MTA, its surface

microhardness was reduced significantly. They further reported that a higher compressive strength occurred with a lower condensation pressure (0.06 MPa), which seems to indicate that even when condensation pressures are controlled to prevent MTA from being extruded into the adjacent tissues, the resultant material will be relatively strong (Nekoofar *et al.* 2007).

To investigate MTA crystal formation in a simulated clinical situation, Tingey *et al.* (2008) analysed the surface characteristics of MTA when set in the presence of foetal bovine serum. They demonstrated that foetal bovine serum affected the dynamics of MTA crystal nucleation and lattice growth suggesting potential effects on the cellular response to the material. However, foetal bovine serum could be considered to provide a poor alternative to human serum and/or whole human blood, and it might not entirely replicate clinical conditions in humans.

In certain clinical applications, MTA might be unintentionally surface contaminated or mixed with blood and/or interstitial fluid. Therefore, the aim of this study was to evaluate the effect of human serum and whole blood exposure on surface microhardness and surface microstructure of white and grey MTA in the short and long term. In addition, the materials were also mixed solely with whole blood, in place of water, to determine whether incorporation of this potential contaminant would affect their physical properties.

Materials and methods

The parameters investigated were surface microhardness (Vickers microhardness) and assessment of surface morphological characteristics using scanning electron microscopy (SEM). The materials investigated were ProRoot[®] MTA Original (Dentsply Tulsa Dental, Johnson City, TN, USA) with LOT number of 05003087 (grey) and ProRoot[®] MTA (Dentsply Tulsa Dental) with LOT number of 083006 (white).

Specimen preparation

Three groups of 10 samples for each type of MTA (60 samples in total) were prepared. The MTA in the first group (10 samples of grey and 10 samples of white MTA) was mixed with whole fresh human blood and exposed to the same fluid during the setting period; it was not mixed with water. The second group was mixed with distilled water and exposed to whole fresh human blood, and the third

was mixed with distilled water and exposed to fresh human serum.

During preparation, 1 g of each type of MTA powder was mixed with 0.33 g of the selected liquid. The powder of each sample was measured and added to an empty plastic capsule together with the correct weight of the selected fluid and a plastic pestle. The encapsulated material was triturated immediately for 30 s at 4500 rpm using an amalgamator (Silamat®; Ivoclar, Vivadent AG, Liechtenstein) as suggested by Nekoofar et al. (2010).

Group 1 (mixed with blood and exposed to blood)

Human blood was obtained by phlebotomy using a 23-gauge needle from a healthy volunteer member of the research group who gave informed consent. All documentation and procedures were approved by the ethical board of the local research review committee in the Faculty of Dentistry, Tehran University of Medical Sciences, Iran. The MTA samples mixed with whole fresh human blood were introduced incrementally into customized borosilicate glass cylindrical moulds that were placed on a glass slab. Each mould, having an internal diameter of 4 mm and height of 6 mm, had been pre-filled with whole blood that was then gently aspirated; therefore, the internal surface of the mould was coated with blood prior to filling (Fig. 1). Each sample was then treated with ultrasonic energy for 30 s at scale 5 using a CPR-2D tip (Obtura Spartan, Fenton, MO, USA) with a Suprasson P5 ultrasonic booster (Satelec, Merignac, France). The tip of the ultrasonic device was placed in the centre of the material and not in contact with the walls or floor of the mould. The extruded material was then removed.

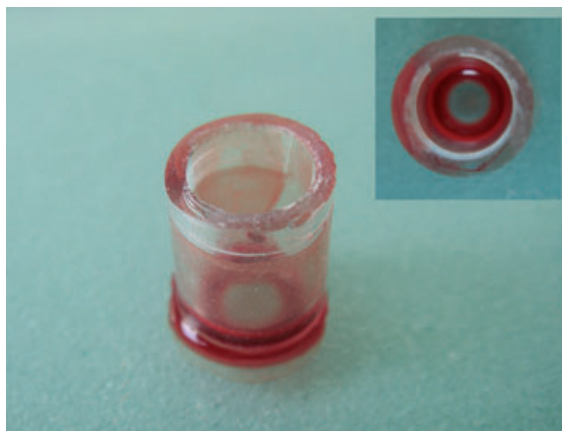


Figure 1 In experimental groups exposed to blood, the internal surface of moulds was coated with blood prior to filling.

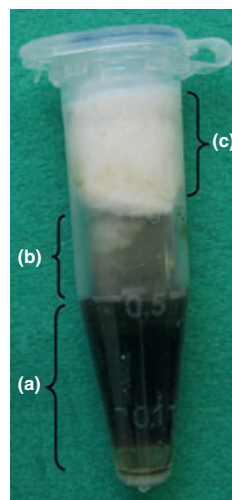


Figure 2 The experimental model. (a) Cotton pellet soaked in whole blood at the bottom of an 1.5-mL Eppendorf tube. (b) Borosilicate glass cylindrical mould containing a mixture of blood and mineral trioxide aggregate (MTA). (c) Damp cotton pellet to maintain humidity.

The filled borosilicate moulds were then placed into a 1.5-mL conical plastic tube (Eppendorf UK Ltd, Cambridge, UK) containing a cotton pellet soaked in whole blood (Fig. 2) and incubated at 37 °C and fully saturated humidity for 4 days.

Group 2 (mixed with water and exposed to blood)

Mineral trioxide aggregate was mixed with distilled water and placed in similar borosilicate glass cylindrical moulds in which the internal surfaces were coated with whole blood prior to placement at 37 °C and fully saturated humidity for 4 days.

Group 3 (mixed with water and exposed to serum)

Human serum was obtained by immediately centrifuging 4 mL of freshly aspirated blood from the same donor for 5 min at 11180 g (10 000 rpm). The upper plasma layer of the centrifuged liquid was gently aspirated into a 5-mL syringe. One gram of the MTA powder was mixed with 0.33 g of distilled water as described in group 2. The samples were then exposed to human serum during setting in the same manner used for the exposure to blood of groups 1 and 2.

Control group

A group of 10 samples for each type of MTA, grey and white (20 samples in total), were used as the control group. One gram of the sample powder was mixed with

0.33 g of distilled water in the method used for the experimental groups. These samples were then exposed only to distilled water during the setting period in the form of a moistened pellet.

Surface microhardness

After 4 days, all samples were removed from the incubator and subjected to a surface microhardness test. The surface of each sample was rinsed with tap water for 60 s to remove surface debris and then wet-polished at room temperature using minimum hand pressure and silicon carbide grinding papers (Buehler-Met®; Agar Scientific Limited, Cambridge, UK) of 320-grit, 600-grit and 1200-grit, respectively. The Vickers surface microhardness of each specimen was then performed in accordance with BS EN 843-4 (2005) using a Micromet 5114 tester (Buehler Ltd, Lake Bluff, IL, USA) with a square-based pyramid-shaped diamond indenter with a full load of 500 g for 30 s at room temperature. This produced a quadrangular depression with two equal orthogonal diagonals in the polished surface of the cement. The angle between the opposite faces of the diamond indenter was 136°. Ten indentations were randomly made on the polished surface of each specimen at separated locations to adjacent indentations or from the specimen periphery. The diagonal produced from the indentation was measured immediately under the microscope, and the Vickers surface microhardness value was displayed on the digital readout. The Vickers hardness (HV) is calculated based on the following formula:

$$HV = \frac{2F \sin \frac{136^\circ}{2}}{d^2} \quad HV = 1.854 \frac{F}{d^2} \text{ approximately}$$

Where F is load (kg^{-1}) and d is the mean of the two diagonals produced by the indenter in millimetres.

After microhardness testing, all samples were immediately replaced into the Eppendorf tubes that contained a cotton pellet soaked in distilled water and placed inside an incubator at 37 °C and fully saturated humidity. After 6 months, the samples were removed from the incubator and subjected to the surface microhardness test using the same methodology.

The mean Vickers surface microhardness values were calculated for each group and subjected to a one-way analysis of variance and a Tamhane *post hoc* test. All analysis was performed using the Statistical Package for the Social Sciences version 16 (SPSS Inc., Chicago, IL, USA).

Scanning electron microscopy

The morphological evaluation by SEM of the different test groups was carried out on the new specimens prepared by the same methodology. The surfaces were sputter-coated with gold using a Polaron Sputter Coater (Quorum Technologies, Newhaven, UK), and specimens were analysed with an EBT1 (Electron Beam Technology) scanning electron microscope (S.E.M. Tech Ltd, Woodbridge, UK). The micrograph images from the SEM analysis revealed the qualitative external microstructure of the various MTA groups. The samples were evaluated and compared in relation to the presence of micro-channels and type of crystal formation.

Results

Microhardness

The microhardness values obtained for the different experimental groups after 4 days and 6 months are displayed in Fig. 3.

After 4 days

The white MTA had a greater microhardness value than grey MTA in every group comparison made. The mean Vickers surface microhardness value of the white MTA control group was 59.91 ± 5.72 . This was significantly greater than the mean surface microhardness value of the grey MTA control group, which was 45.97 ± 3.78 ($P < 0.00001$). There was a statistically significant difference between the control groups and all the experimental groups ($P < 0.00001$).

In addition, the white MTA mixed solely with blood and the white MTA exposed to serum, compared to the other experimental groups, had the highest Vickers microhardness values at 36.52 ± 4.60 and 35.32 ± 6.78 , respectively ($P < 0.00001$). In contrast, the grey MTA mixed with blood had the lowest microhardness value out of all the experimental groups at 18.44 ± 3.92 ($P < 0.00001$).

After 6 months

The microhardness values of the samples after 6 months had a similar pattern to those values obtained after 4 days. The control groups had significantly greater microhardness values than the experimental groups, and white MTA had a significantly greater microhardness than grey MTA in every group ($P < 0.00001$).

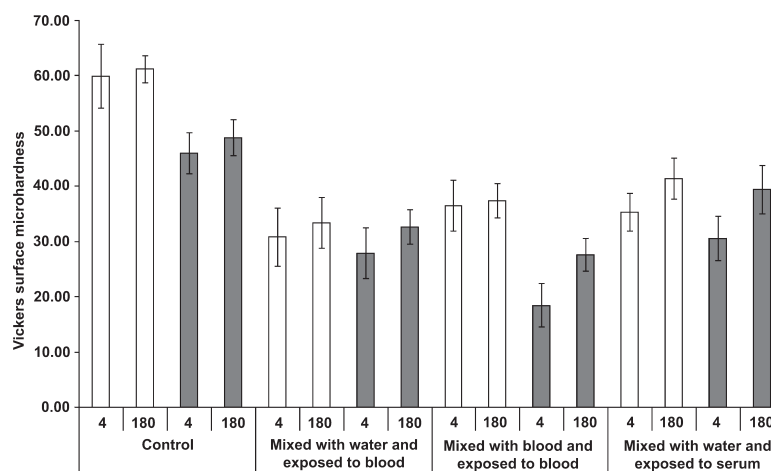


Figure 3 Vickers surface microhardness values after 4 and 180 days. Presence of 4 and 180 refers to the number of incubation days. The colour of bars corresponds with the types of mineral trioxide aggregate (MTA) used (white and grey).

There was no significant difference between the microhardness values after 4 days and 6 months within the sample groups, apart from the samples of grey MTA mixed with blood and the grey MTA exposed to human serum. Both these samples had a significantly increased microhardness value after 6 months ($P < 0.00001$). In accordance with the results obtained after 4 days, grey MTA mixed with blood had the lowest microhardness value after 6 months. However, both white and grey MTA exposed to serum had the highest microhardness values within the experimental groups at 41.40 ± 7.52 and 39.41 ± 8.75 , respectively ($P < 0.00001$).

SEM

The results of the SEM can be seen in Figs 4–6. Across all groups, no discernable difference was obvious between the samples of grey MTA and white MTA. In the control groups, samples revealed notable crystalline characteristics when compared to experimental groups (Fig. 6). Such formations included laminar, plate-like crystals with well-defined edges, which were embedded in a porous and rough crystalline matrix. Additionally, acicular crystals, characteristic of hydrated calcium sulphoaluminate (ettringite) (Gemelli *et al.* 2004), were observed in jagged clusters as well as longer structures that spanned and interlinked other crystals (Figs 4 and 5).

The surfaces of all experimental samples had a noticeably different appearance than control specimens (Fig. 6). In the experimental groups, there was a general lack of angular crystal formations and an absence of both the jagged clusters and longer forms of the interlinking acicular ettringite crystals (Fig. 6iii–viii). They also exhibited a more globular crystalline

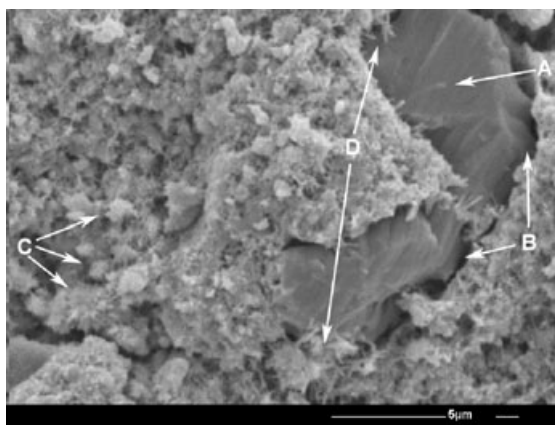


Figure 4 Scanning electron microscope image of a control sample of mineral trioxide aggregate (MTA) mixed with and exposed to distilled water. A large laminated plate-like crystal with well-defined edges (A) was embedded in a rough crystalline matrix containing micro-channels (B). Acicular crystals were seen in clusters of spiky ball formations (C) as well as longer spanning forms (D) radiating from the rough matrix.

matrix than control samples. However, in samples mixed with water and exposed to blood, it was possible to see a more angular matrix than the other experimental groups (Fig. 6iii, iv).

Discussion

Mineral trioxide aggregate is a hydraulic cement and consists of fine hydrophilic particles that on contact with water sets to a hard composition through the creation of a colloidal gel (Lee *et al.* 2004, Camilleri *et al.* 2005). The first commercial product was

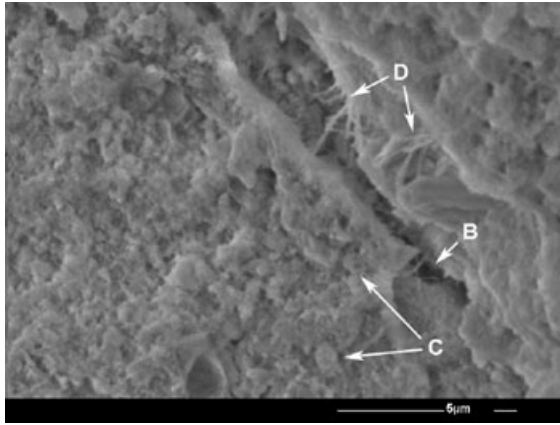


Figure 5 Scanning electron microscope image of a control sample of mineral trioxide aggregate (MTA) mixed with and exposed to distilled water. A rough crystalline matrix was seen to contain micro-channels (B) and two forms of acicular crystals; clusters of jagged shapes (C) and needle-like shapes (D) interconnecting other crystals.

launched as ProRoot[®] MTA (Dentsply Tulsa Dental). The initial product was a grey-coloured material, but as this initial formulation was thought to stain teeth (Antunes Bortoluzzi *et al.* 2007), a white version was subsequently developed. Recently, another commercially available cement was launched as MTA-Angelus (Angelus, Londrina, Brazil). Both grey and white versions of MTA-Angelus are available. White and grey MTA have a similar composition, although there are differing levels of aluminium, magnesium and iron (Asgary *et al.* 2005). The reduced level of iron in white MTA is reported to be the most significant difference between the two types (Camilleri *et al.* 2005, Asgary *et al.* 2006, Song *et al.* 2006).

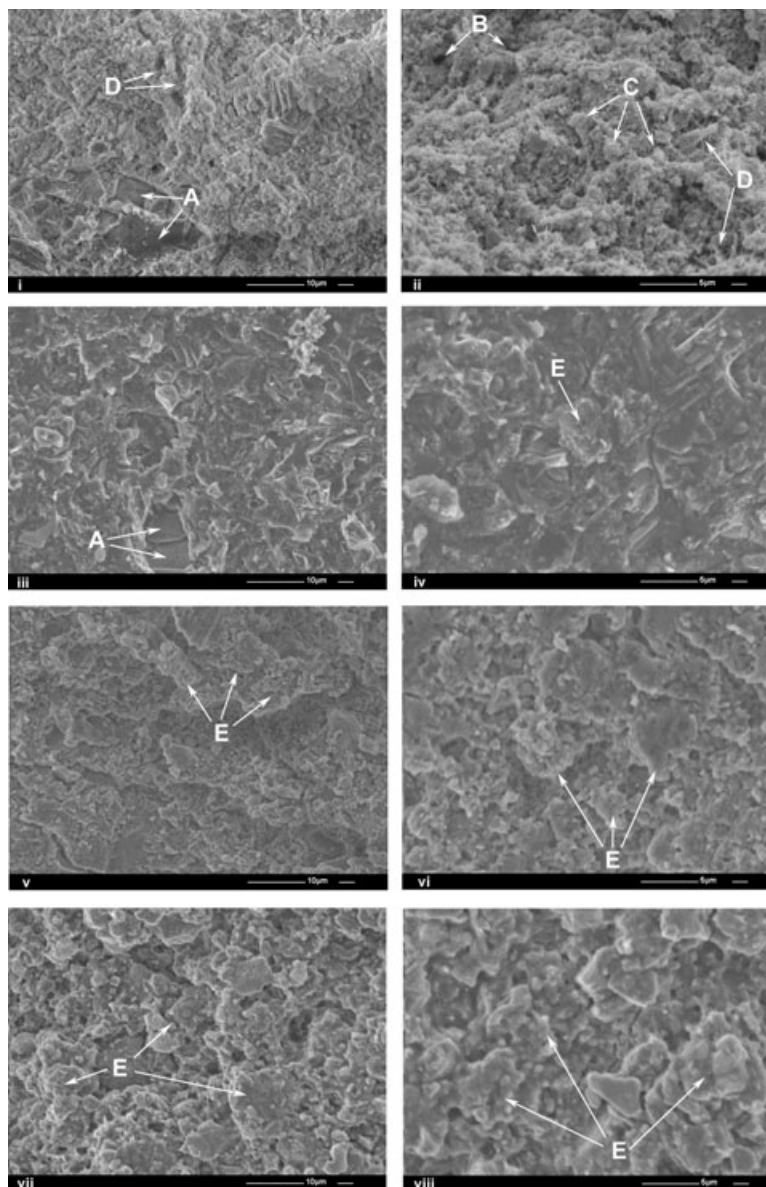
Mineral trioxide aggregate has numerous properties that are beneficial clinically, such as biocompatibility (Vajrabhaya *et al.* 2006), antibacterial nature (Al-Hezaimi *et al.* 2006), potential to seal against bacterial penetration (Adamo *et al.* 1999) and the ability to set in a moist environment (Lee *et al.* 2004). Therefore, the material is used primarily in the repair of root perforations, vital pulp therapies and for root-end fillings (Abedi & Ingle 1995). In the course of use, the material is frequently in close contact with blood, and this fluid might even mix with the body of the cement owing to the specific clinical conditions. Therefore, it is imperative to investigate the setting reaction of the material in an environment where blood is present and explore any potential changes to the crystalline micro-

structure and surface microhardness as a result of this contamination.

The surface microhardness of the material might provide an indication of the degree to which the material has undergone hydration during the setting reaction (Lee *et al.* 2004, 2007, Camilleri 2007, 2008, Nekoofar *et al.* 2007). In addition, surface microhardness can be measured using a nondestructive test, allowing further investigation and exploration of any changes to this property over time (Igarashi *et al.* 1996). In this study, the surface microhardness of MTA was evaluated after 4 days and 6 months with no significant difference in values between the two time periods, for any group. However, the results did demonstrate that blood contamination had a detrimental effect on the surface microhardness of MTA. It could be suggested that testing the compressive strength of MTA might provide additional information. However, this is a destructive investigation and cannot be repeated on the same sample over time as is possible for the testing of surface microhardness.

In addition to the testing of surface microhardness, the surface microstructure of the samples was examined using a SEM. Owing to the nature of this investigation, it was not possible to use a quantitative method of comparison. However, descriptive analysis revealed that the samples contaminated with human fluids (blood and/or serum) had an altered surface microstructure that was devoid of the acicular crystal formations, characteristic of hydrated calcium sulphoaluminate (ettringite) (Gemelli *et al.* 2004), that were prominent in the control groups (Fig. 6). These findings are similar to those reported by Lee *et al.* (2004), Namazikhah *et al.* (2008) and Kayahan *et al.* (2009) where MTA was exposed to an acidic environment. However, blood is known to have a near-neutral pH (Kellum 2000), so the changes to the surface characteristics of the experimental samples in the present study cannot be attributed to acidity. It was observed that the acicular crystals had the appearance of interlinking other crystals in MTA (Lee *et al.* 2004, Namazikhah *et al.* 2008), as has also been described in Portland cement (Gemelli *et al.* 2004, Stutzman 2004). Therefore, it can be hypothesized that the absence of the acicular crystals could have an adverse effect on surface microhardness. The potential role of interlinked needle-like crystals in forming inter-crystal bonds and improving mechanical behaviour of the Portland cement were demonstrated by Ismail *et al.* (2002). Further chemical analysis of MTA to evaluate the function of these crystals is recommended.

Figure 6 Scanning electron microscope images comparing mineral trioxide aggregate (MTA) surfaces under two levels of magnification, higher magnifications are shown on the right. A prominent presence of both types of acicular crystal formations, jagged clusters (C) and long spanning shapes (D), were seen in control groups (i, ii) that were absent in all experimental groups (iii–viii). In general, a globular matrix (E) with less angular crystal formations was seen in experimental groups (iii–viii), when compared to the angular crystal formations (A) embedded in the rougher matrix of control samples containing more micro-channels (B).
 (A) Lamellar plate-like crystals.
 (B) Cross-section of micro-channels.
 (C) Jagged balls of acicular crystal formations.
 (D) Long needle-like acicular crystals radiating from the rough matrix.
 (E) Globular matrix.
 (i, ii) Control groups mixed with water and exposed to water.
 (iii, iv) Experimental groups mixed with water and exposed to blood.
 (v, vi) Experimental groups mixed with water and exposed to serum.
 (vii, viii) Experimental groups mixed with blood and exposed to blood.



It is necessary to attempt simulation of the clinical environment during the placement of MTA. This modelling of the clinical conditions may be achieved by the use of human or animal blood and/or serum. The use of serum, in comparison with whole blood, has the advantage of no propensity to clot outside the body and, therefore, might provide a more accurate representation of the conditions of bioactivity found during clinical placement. However, it is likely that the fluid that remained after fresh human blood clotting had a similar composition and properties to the human serum obtained by centrifugation.

Tingey *et al.* (2008) used foetal bovine serum to simulate the potential effect of clinical contamination on the crystal microstructure of MTA. They concluded that MTA exposure to foetal bovine serum resulted in differing crystal formation during the setting reaction. Research studies have more clinical relevance if fresh human blood products are used. In the present study, human blood and serum were utilized as the model for clinical contamination. A pilot experiment was initially carried out to confirm the feasibility of the chosen method before commencing the study on the relatively large scale planned. For this pilot investigation, the

human serum was sourced from the National Blood Transfusion Service, a choice made on the basis of low cross-infection risk and relative ease of availability. This initial experiment was unsuccessful, as the MTA samples mixed with the supplied serum did not set to the usual consistency and the surface was too soft to be investigated. It was also possible to penetrate the entire depth of the samples with a dental probe. This unexpected outcome was investigated by conducting the same experiment successfully with fresh blood and serum obtained in the method described for the main research experiment. It was, therefore, deduced that the incomplete hydration of MTA resulted from the addition of a citrate anticoagulant, by the Blood Transfusion Service, to the blood sample that was centrifuged to obtain the serum used. This addition is a standard practice by the Blood Transfusion Service (James 2005).

The results of this study after 6 months indicate that within the experimental groups, MTA exposed to serum had the highest microhardness values. This difference after 6 months might be explained by the absence of clotting in the serum samples in comparison to blood. In the study by Tingey *et al.* (2008), the foetal bovine serum used was first frozen by the manufacturer and then thawed for use. According to the manufacturer, there is the potential problem of flocculence on thawing owing to the denaturation of serum lipoproteins (http://itools.nvitrogen.com/content/sfs/brochures/B-066802-Sera_Bro.pdf). Regular mixing during thawing is recommended, and the possible need for centrifugation and refiltration is emphasized. Therefore, it is possible to hypothesize that the use of frozen and thawed blood products might have an effect on the biochemistry of the product compared to fresh material and the potential for alteration of any effect on MTA during setting. Tingey *et al.* (2008) allowed 24 h of incubation of the MTA samples before the examination of the surface microstructure. It could be suggested that the hydration of MTA may not have been entirely complete at this time. In this study, the samples were incubated for 4 days before the initial microhardness testing. This time period is in accordance with Vanderweele *et al.* (2006), Song *et al.* (2006), Nekoofar *et al.* (2007) and Kayahan *et al.* (2009) who suggested that MTA should be left for at least 72–96 h to decrease the likelihood of displacement and increase surface microhardness and compressive strength. Bodanezi *et al.* (2008) also reported that the solubility of MTA decreases after 72 h. In addition, Sluyk *et al.* (1998) concluded that for achieving the desirable sealability, MTA should be untouched for at least 3 days when used to repair root perforations.

The results of this study demonstrated that the surface microhardness value of white MTA was considerably greater than that of grey MTA when mixed and set within the ideal, recommended conditions, as demonstrated by the HV results of the control groups. It is interesting that white MTA had greater hardness than grey MTA in every group comparison made. As might perhaps be expected, grey MTA had a reduced surface microhardness when mixed with blood, when compared to only exposure. However, quite unexpectedly, the white MTA mixed with blood had a greater hardness than the white MTA only exposed to blood, in addition to being harder than all other experimental groups. The reason for this difference is unclear, and further investigation would be beneficial.

All of the experimental groups in contact with blood had a reduced hardness in comparison to the control groups. Therefore, it is possible to make a supported recommendation that clinicians should attempt to control bleeding when placing MTA in any clinical situation. In addition, it could be suggested that white MTA would be a more suitable choice than grey if the management of bleeding should prove to be difficult, which might occur during the repair of root perforations and the placement of root-end fillings. It can also be ascertained that the samples of white and grey MTA mixed with human serum had reduced levels of hardness compared to the control groups. This reduction in microhardness follows the trend for the samples of MTA mixed with blood. In support of this finding, Tingey *et al.* (2008) reported that the presence of serum affected the setting reaction of MTA after examining the surface microstructure of MTA samples.

Other studies have reported that the setting of MTA is adversely affected by a number of environmental factors. These include an acidic pH (Lee *et al.* 2004), an alkaline environment (Saghiri *et al.* 2008) and over-condensation during placement (Nekoofar *et al.* 2007). However, the most important issue to acknowledge is whether the adverse effect on the properties of MTA demonstrated by this study and others has a detrimental consequence for the material after placement and its subsequent longevity. Clearly, further research is necessary in this area.

Conclusions

White MTA had a greater surface microhardness than grey MTA in all the experimental groups and also in the control group. Blood contamination had a

detrimental effect on the surface microhardness of MTA and caused a change in the surface microstructure. Therefore, it might be suggested that when using MTA, attempts should be made to control bleeding. If contamination is unavoidable, white MTA might be a more suitable choice as the results show this material to be less significantly affected than grey MTA. The surface microhardness values of the MTA samples after 6 months were similar to those after 4 days. Further research into the significance of blood contamination on the outcome of MTA applications in the form of a clinical trial would be beneficial.

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