The effect of blood contamination on the compressive strength and surface microstructure of mineral trioxide aggregate

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Abstract

Aim To investigate the effects of whole, fresh human blood contamination on compressive strength and surface microstructure of grey and tooth-coloured mineral trioxide aggregate (MTA).

Methodology The materials investigated were grey ProRoot® MTA Original (Dentsply Tulsa Dental, Johnson City, TN, USA) and tooth-coloured ProRoot® MTA (Dentsply Tulsa Dental). Three groups of 10 custom-made cylindrical moulds (internal dimensions 6 ± 0.1 mm length and 4 ± 0.1 mm diameter) were filled with tooth-coloured MTA. In the control group, MTA was mixed with water and exposed to water. In the second group, MTA was mixed with water and exposed to whole, fresh human blood. In the third group, MTA was mixed with and exposed to whole, fresh human blood. These three groups were then duplicated using grey MTA, creating a total of 60 samples. A predetermined amount of MTA and appropriate liquid were triturated in a plastic mixing capsule then subjected to ultrasonic energy after placement in the moulds. After 4 days of incubation, specimens were subjected to compressive strength testing. The surface microstructure of one extra specimen in each group was examined using scanning electron microscopy. Data were subjected to a two-way ANOVA.

Results Regardless of MTA type, the mean compressive strength values of both experimental groups, which were in contact with blood, were significantly less than that of the control groups ($P < 0.0001$). In experimental groups in which MTA was mixed with water and exposed to blood, there was a significant difference ($P < 0.0001$) in compressive strength between tooth-coloured MTA (30.37 ± 10.16 MPa) and grey MTA (13.92 ± 3.80 MPa).

Conclusion When blood becomes incorporated into MTA, its compressive strength is reduced. In clinical situations in which blood becomes mixed with MTA, its physical properties are likely to be compromised.

Keywords: blood contamination, compressive strength, Mineral Trioxide Aggregate, MTA, SEM.

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Introduction
Perforation of the root canal walls or bifurcation region can be a complication of access cavity preparation, root canal shaping or preparation of post-holes (Ingle et al. 2007). Immediate repair of a perforation with a biocompatible material is recommended for achieving the optimum outcome (Pitt Ford et al. 1995, Fuss & Trope 1996). In addition to biocompatibility, an ideal repair material should be antibacterial (Al-Hezaimi et al. 2006), be capable of close adaptation to root canal walls (Shokouhinejad et al. 2010), be radiopaque (Camilleri 2009) and nonresorbable (Ghoddusi et al. 2007). Mineral trioxide aggregate (MTA) possesses
these properties and can be differentiated from other root repair materials by its additional ability to conduct cementum and bone formation over its surface (Zhu et al. 2000). It can also set in a wet environment (Torabinejad et al. 1995, Koh et al. 1998). These properties are also desirable for a root-end filling material (Torabinejad & Chivian 1999), for pulp capping materials used during vital pulp therapies (Pitt Ford et al. 1996), for apexification of immature teeth with necrotic pulps (Witherspoon & Ham 2001), for the nonsurgical repair of invasive cervical root resorption (Schwartz et al. 1999) and for the repair of horizontal root fractures (Mc Cabe 2003).

In these clinical applications, blood comes into contact with and often becomes incorporated into MTA during or after its placement, and this contamination might have a detrimental effect on its physical properties. The effect of blood contamination on dye leakage of an initial prototype of MTA was investigated in an ex vivo endodontic surgery model, which concluded that blood contamination had no significant effect on dye leakage (Torabinejad et al. 1994). In another laboratory study, Martell & Chandler (2002) compared electrochemical and dye leakage of Super-EBA, IRM and MTA in root-end cavities after immersion for 24 h in defibrinated horse blood. The authors concluded that MTA was associated with less leakage than the other materials. In an animal study, the same materials along with a zinc oxide and eugenol (ZOE) base material were passively exposed to blood in root-end cavities of mandibular premolar teeth in dogs (Bernabe et al. 2005). The materials used in that study, including MTA, all had similar effects on the healing process, except for ZOE that resulted in a significantly worse outcome. VanderWeele et al. (2006) placed MTA in simulated root perforations, which had previously been contaminated with human blood and found significantly higher resistance to displacement for uncontaminated samples of MTA after 7 days. In a similar study, Montellano et al. (2006) used an ex vivo model to compare the effects of saline, human blood and saliva contamination on tooth-coloured MTA when used to repair simulated root perforation sites. They found no significant difference in bacterial suspension penetration when comparing blood contaminated and uncontaminated groups, although the saliva-contaminated groups resulted in significant bacterial penetration.

Because of biosafety issues and difficulties in obtaining fresh human blood, Tingey et al. (2008) emulated the exposure of MTA to human tissue fluid by exposing samples to foetal bovine serum (FBS) and investigated the exposed surface microstructure with scanning electron microscopy. They demonstrated marked morphologic differences in the surfaces of MTA samples when exposed to either distilled water or FBS. In the critique of their work, they suggested future studies in this area of research should use more relevant experimental models.

The aim of this study was to investigate the effects of fresh human blood contamination on compressive strength and surface microstructure of grey ProRoot® MTA Original (Dentsply Tulsa Dental, Johnson City, TN, USA) and tooth-coloured ProRoot® MTA (Dentsply Tulsa Dental). Whole, fresh human blood was used to contaminate MTA samples, rather than the various human blood substitutes that have been used in other similar studies.

**Methods and materials**

The materials investigated were grey MTA (ProRoot® MTA Original, LOT number 05003087) and tooth-coloured MTA (ProRoot® MTA, LOT number 083006).

Whole, fresh human blood was collected from a healthy consented volunteer member of the research group by a trained individual in accordance with Helsinki ethical principles for medical research involving human subjects (2001) and approved by a panel from the School of Dentistry, Cardiff University Ethical Committee. Sixty custom-made polytetrafluoroethylene (PTFE) cylindrical moulds (internal dimensions 6 ± 0.1 mm length and 4 ± 0.1 mm diameter) were randomly allocated to six groups, prior to filling with MTA. Tooth-coloured MTA was used in groups 1, 3 and 5, and grey MTA used in groups 2, 4 and 6. The groups consisted of:

- Groups 1 and 4 – MTA mixed with water and exposed to water as control groups;
- Groups 2 and 5 – MTA mixed with water and exposed to blood;
- Groups 3 and 6 – MTA mixed with blood and exposed to blood.

Mixing of MTA was standardized by placing 1 g of either type of MTA and 0.33 g of appropriate liquid in a plastic mixing capsule with a plastic pestle to facilitate trituration (Nekoofar et al. 2010). The plastic capsules were immediately sealed and loaded into a Promix TM amalgamator (Dentsply Caulk, York, PA, USA) and triturated at 4500 revolutions min⁻¹ for 30 s. The PTFE cylindrical moulds were then placed on a glass slab and filled with the resultant MTA slurries using a
spatula with minimal pressure; 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). The ultrasonic tip was moved throughout the MTA slurry without touching either the mould walls or the glass slab, whilst being activated for 30 s at power scale 5. Cylindrical moulds filled with MTA were then wiped over the glass slab to remove excess MTA slurry from each end of the specimens.

In the test groups (3, 4, 5 and 6), before placement of the MTA slurry, the cylindrical moulds were filled with whole, fresh human blood that was then removed by aspirating with a syringe to leave a coating of blood on the inner wall of the moulds. All samples were placed in sealed 1.5-mL Eppendorf tubes following placement of the appropriate liquid medium used to expose the lower surface of MTA specimens. A moist cotton pellet was then placed above the moulds but not in contact with the MTA surface to produce a fully saturated humid atmosphere (Fig. 1). They were then incubated at 37°C for 4 days in accordance with Nekoofar et al. (2010). The samples were removed from the incubator, and the end surfaces were polished with 1200-grit fine-grain sandpaper (3M, St Paul, MN, USA) to dimensions of 6 ± 0.1 mm length and 4 ± 0.1 mm diameter, in accordance with ISO 9917-1:2003 standards. The MTA samples were then removed by cutting vertically through the wall of the moulds using a disposable surgical scalpel blade No.15, whilst taking care not to damage the MTA samples (Fig. 2). Following removal from the moulds, all samples of MTA were inspected visually to ensure they had no voids or flaws before being subjected to the compressive strength test.

**Compressive strength test**

To test for compressive strength, samples were placed vertically on the steel plate of a universal testing machine (Lloyd LR MK1 machine: Lloyd Instruments, Fareham, UK) towards which a calibrated steel cross head plate moved at a speed of 1 mm min⁻¹. When both planes were in contact with the samples, the compressive load was recorded until a loading failure point was reached. This loading failure was used to calculate the compressive strength of the MTA samples using the following equation:

$$CS = \frac{4P}{\pi d^2}$$

where CS is compressive strength, P(N) is loading failure and d (mm) is the diameter of the cylindrical samples.

**Scanning electron microscopy**

To examine the MTA surface characteristics, an additional MTA sample from each of the six groups were created using the same methods as described earlier. The surfaces of the MTA samples exposed either
to water or blood were sputter-coated with gold (Polaron Sputter Coater; Quorum Technologies, Newhaven, UK) and then analysed with an EBT1 (Electron Beam Technology) Scanning Electron Microscope (S.E.M. Tech Ltd, Woodbridge, UK). Images generated from the SEM were then evaluated in a qualitative manner to describe the characteristics of the surface microstructure in terms of the crystal formations, by two evaluators who were not informed of the image origin.

The mean compressive strengths, confidence intervals and standard deviation values were calculated for each group and analysed using two-way ANOVA as the data was normally distributed. All analysis was performed using the statistical package of social science version 16 (SPSS Inc., Chicago, IL, USA).

Results

A summary of the results of the compressive strength tests are shown in Fig. 3. The compressive strengths of both experimental groups, which were in contact with blood, were significantly less than that of the control groups ($P < 0.0001$).

Effect of blood contamination

Regardless of MTA type, when samples were mixed with and exposed to blood, a significant difference was found in mean compressive strength values when compared to control groups and groups only exposed to blood ($P < 0.0001$). In addition, when samples were mixed with water and exposed to blood, the mean compressive strength value was significantly lower than that of the control group ($P < 0.0001$).

Tooth-coloured MTA

A significant difference was found between the control (group 1) and experimental specimens (groups 3 and 5) of tooth-coloured MTA ($P < 0.0001$). The lowest mean compressive strength value was recorded for MTA specimens, which were both mixed with and exposed to whole, fresh human blood (group 5) (1.21 ± 0.31 MPa). The highest mean compressive strength value was recorded for the control specimens (group 1) (71.36 ± 24.81 MPa).

Grey MTA

A significant difference was found between the control (group 2) and experimental specimens (groups 4 and 6) of grey MTA specimens ($P < 0.0001$). The lowest mean compressive strength value was recorded for MTA specimens, which were both mixed with and exposed to whole fresh human blood (group 6) (1.66 ± 0.25 MPa). The highest mean compressive strength value was recorded for the control specimens (group 2) (49.73 ± 14.77 MPa).

Effect of MTA type

In the optimal conditions of the control specimens (groups 1 and 2), the mean compressive strength value of tooth-coloured MTA (71.36 ± 24.81 MPa) was higher than that of grey MTA (49.73 ± 14.77 MPa), but the difference was not significant. The only significant difference in mean compressive strength values when comparing tooth-coloured MTA and grey MTA was found between group 3 (30.37 ± 10.16 MPa) and group 4 (13.92 ± 3.80 MPa), which were
both mixed with water and exposed to blood ($P < 0.0001$).

**SEM**

The surface microstructure of MTA specimens revealed substantial differences in crystalline formations between control and experimental groups. However, when comparing grey and tooth-coloured MTA within all groups, there were no discernable differences in surface microstructure. In both control groups, a wide variety of distinctive crystalline formations around cross-sections of micro-channels were seen (Figs 4 and 5i). Such formations included angular and laminar crystals along with two main forms of acicular crystals characteristic of hydrated calcium sulphaaluminate (ettringite) (Gemelli et al. 2004). These characteristic formations include jagged or spiky ball-like clusters and bundles of longer spanning structures that interlink other crystals (Fig. 4). All experimental, blood-contaminated, groups had more globular formations, rather than the angular crystals seen in the control groups. In addition, these experimental groups also demonstrated a clear lack of either types of acicular crystal that were prominent in the control groups.

**Discussion**

According to the US patent 5,415,547, the principle component of MTA is Portland cement (Torabinejad & Dean 1995), which consists of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tricalcium aluminoferrite and calcium sulphate (Islam et al. 2006). In addition to these principle components, MTA also contains bismuth oxide to make it radiopaque (Coomaraswamy et al. 2007). Portland cement, and its dental derivative, MTA, are hydraulic cements, which are able to set and harden under water (Camilleri 2007). This is an advantageous property of MTA when moisture control is difficult. Other beneficial properties of MTA include biocompatibility and its close adaptation to canal walls that have produced promising outcomes in a wide range of applications within endodontics (Main et al. 2004, Ghoddusi et al. 2007, Saunders 2008, Mente et al. 2009). Some disadvantageous features of MTA include difficult handling properties (Hsieh et al. 2009) and temperamental setting characteristics (Ber et al. 2007), which could be because of low environmental pH (Torabinejad & Chivian 1999, Namazikhah et al. 2008, Nekoofar et al. 2009) and/or insufficient hydration (Camilleri 2007).

**Figure 4** Scanning electron microscope image of a control sample of MTA mixed with and exposed to distilled water. Large laminated plate-like crystals with well defined edges (A) were 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. Three levels of magnification are shown with the highest at the bottom.
In this study, the compressive strength of MTA was used as a measure of the hydration process (Torabinejad et al. 1995, Nekoofar et al. 2007, Kayahan et al. 2009).

According to ISO 9917-1 (2003) standards, the use of a split mould design, made of stainless steel or a material that will not be affected by the cement, has been advised. There have been a variety of methods used to form cylindrical MTA specimens for compression testing. In this study, PTFE plastic cylindrical moulds were used to form 6 × 4 mm diameter MTA samples. In other investigations of MTA compressive strength, one-piece plastic cylindrical moulds (Kogan et al. 2006), one-piece polycarbonate cylindrical moulds (Nekoofar et al. 2007), plastic split moulds (Holt et al. 2007) and stainless steel split moulds (Kayahan et al. 2009) have been used. Because of the expansion of MTA during its hydration, Holt et al. (2007) reported difficulties during the removal of specimens from moulds without exerting excessive force on the material prior to testing. Holt et al. (2007) used a two-part split mould design to create MTA samples for compression testing and reported that samples required moderate force to allow removal, which resulted in fracture failure of some samples prior to testing. In a pilot study prior to this study, single-piece borosilicate glass moulds were used to form MTA samples, which required a high push-out force to remove them for testing and resulted in multiple sample fractures. Therefore, in this study, a new method of MTA sample formation was developed that involved careful removal of two opposing sections of the cylindrical mould walls to reduce retention of the MTA samples (Fig. 2). This novel method minimized the forces on MTA samples prior to compression testing that might otherwise introduce confounding variables.

In the majority of endodontic applications, MTA slurry comes into contact with blood and in the extreme might become mixed with blood during placement. The results of this study revealed that both these events adversely alter the compressive strength of MTA. In addition, in the blood-contaminated groups, an absence of acicular crystals, characteristic of hydrated calcium sulfoaluminate (ettringite) (Gemelli et al. 2004, Stutzman 2004), that have a potential role in forming inter-crystal bonds (Ismail et al. 2002), was demonstrated by SEM (Fig. 5ii, iii). Accordingly, it can be suggested that blood contamination is a likely cause for encountering unset MTA at a subsequent evaluation appointment. According to the Manufacturer’s instruction manual (A0405) Dentsply, Tulsa Dental, Johnson City, TN, USA, if unhardened MTA is encountered at the second appointment, the material should be rinsed out and replaced. However, in surgical applications, MTA cannot be examined for setting, which could potentially result in unfavourable clinical outcomes. For better understanding of the clinical behaviour of the material, particularly when it cannot be examined at a later appointment, further investigations into the effect of blood contamination on the physical properties of MTA are required.

In this study, in an attempt to replicate the clinical situation in which blood becomes incorporated into MTA, the effect of whole, fresh human blood contamination on compressive strength and surface microstructure of two types of MTA was investigated. Whole, fresh human blood was chosen to contaminate MTA rather than substitutes such as defibrinated horse blood (Martell & Chandler 2002), simulated human plasma fluid (Coleman et al. 2007), phosphate-buffered saline (PBS) (Bozeman et al. 2006, Gandolli et al. 2009) or FBS (Tingey et al. 2008). The advantage of using fresh, human blood is that it more closely replicates the human clinical situation. However, experiments involving whole, fresh human blood present difficulties such as ethical considerations, biohazard issues and obtaining sufficient volumes of blood over a prolonged period of time without the addition of anticoagulant agents. The importance of using fresh blood was highlighted by pilot studies using human blood provided by the National Blood Transfusion service, which contained a citrate anticoagulant agent. In these pilot tests, after mixing MTA powder with citrate-treated blood, the slurries remained unset, making compressive strength testing unpractical.

In this study, to exaggerate the blood contamination of MTA, as might occur in some clinical applications, grey and tooth-coloured MTA powders were first mixed with, then exposed to whole, fresh human blood. In the other experimental groups, the two types of MTA powders were mixed with distilled water and exposed to whole, fresh human blood to most closely simulate the clinical conditions. In the control groups, the two types of MTA powder were mixed with and exposed to distilled water.

Holt et al. (2007) and Watts et al. (2007) reported that while MTA generally had a greater compressive strength value than grey MTA. This trend was also observed in this study; however, this pattern was only statistically significant between groups 3 and 4. The MTA samples both mixed with and exposed to whole,
A prominent presence of both types of acicular crystal formations, jagged clusters (C) and long spanning shapes (D), were seen in the control group (i) that were absent in all experimental groups (ii & iii). A globular matrix (E) with less angular crystal formations (F) was seen in experimental groups (ii & iii), when compared to the angular crystal formations (A) embedded in the rougher matrix of control samples containing more micro-channels (B). (A) Laminar 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. (F) Large crystals with rounded edges embedded in globular matrix. (i) Control group- MTA mixed with water and exposed to water. (ii) Experimental group- MTA mixed with water and exposed to blood. (iii) Experimental group- MTA mixed with blood and exposed to blood.

Figure 5 Scanning electron microscope images comparing MTA surfaces from control and experimental groups under the same level of magnification. A prominent presence of both types of acicular crystal formations, jagged clusters (C) and long spanning shapes (D), were seen in the control group (i) that were absent in all experimental groups (ii & iii). A globular matrix (E) with less angular crystal formations (F) was seen in experimental groups (ii & iii), when compared to the angular crystal formations (A) embedded in the rougher matrix of control samples containing more micro-channels (B). (A) Laminar 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. (F) Large crystals with rounded edges embedded in globular matrix. (i) Control group- MTA mixed with water and exposed to water. (ii) Experimental group- MTA mixed with water and exposed to blood. (iii) Experimental group- MTA mixed with blood and exposed to blood.

The groups containing samples of MTA mixed with distilled water and only surface-exposed to whole, fresh human blood also had compressive strength values significantly lower than the control groups. These results suggest that the further blood becomes incorporated into MTA, the more the compressive strength of the material is reduced. Haemoglobin or whole animal blood has been used in Portland cement as an air entrainment admixture to increase porosity (Remadnia et al. 2009). Jasiczak & Zielinski (2006) mixed powdered red blood cells taken from pigs and cows with Portland cement and demonstrated that even small amounts of the red blood cell powder resulted in reduced compressive strength and prolonged setting time of the cement. These findings have been explained by the air entrainment properties of blood proteins that affected the porous microstructure of cements (Remadnia et al. 2009). The air entrainment effects of blood on cement (Jasiczak & Zielinski 2006) and the resultant increased porosity (Remadnia et al. 2009) most likely explain the results of this study, which demonstrated a decreased compressive strength of blood-contaminated MTA. These findings are in accordance with Hesaraki et al. (2006) who showed an increased porosity of calcium phosphate cement when mixed with an air entrainment admixture. Future studies should look at the porosity of blood-contaminated MTA to evaluate the effects of air entrainment, mixing and handling techniques on compressive strength.

At the microstructure level, the blood-contaminated groups demonstrated different crystal morphology. Evaluation of the SEM images revealed a distinct lack of acicular crystals in all groups exposed to or mixed with whole, fresh human blood, when compared to control samples (Fig. 5ii, iii). Ismail et al. (2002) suggested that the bonds between particles of hydrated
Portland cement were created by a dense meshwork of acicular crystal formation that radiate from the cement particles. Stutzman (2004) evaluated the microstructure of hydraulic cement using SEM and X-ray micro-analysis and concluded that the interlinking crystal phase was composed of tricalcium aluminate and/or tetracalcium aluminoferrite. Therefore, in this study, the lower compressive strength values of the groups contaminated with blood is most likely explained by the lack of interlinking acicular crystals. These changes in acicular crystal microstructure upon blood contamination might be the cause of one of the most important disadvantages of MTA, in that in some instances MTA slurries might remain unset and, ideally, should be replaced (Manufacturer’s instruction manual A0405). The characteristic lack of interlinking acicular crystals has also been reported following MTA exposure to acidic conditions (Lee et al. 2004, Namazikhah et al. 2008, Kayahan et al. 2009), which might replicate the clinical environment of infected tissues that have a lower pH than normal (Nekoofar et al. 2009). In this study, a similar lack of acicular crystals was observed despite the fact that the pH of healthy blood is slightly alkaline (pH 7.4). Future studies should attempt to determine the importance of the tricalcium aluminate and/or tetracalcium aluminoferrite crystal phases.

In addition to microstructural changes, uneven hemispherical expansion of MTA samples at cylindrical mould ends was noted in all blood-contaminated groups. The most notable expansions were seen in the experimental groups mixed with and exposed to whole, fresh human blood. However, because of the uneven hemispherical expansion of MTA, precise dimensional measurements were unpractical. Storm et al. (2008) have also described the expansion of MTA when allowed to hydrate in a salt solution, which was used to simulate the in vivo environment. Gandolfi et al. (2009) examined the expansion of MTA when exposed to water, PBS and a mixture of PBS and FBS to replicate the tissue fluids encountered clinically. They found expansion of MTA exposed to the PBS and FBS mixture was less than that of PBS or the water control. They speculated that the proteins in tissue fluids adsorb onto the surface of MTA and block porosities, thus retarding hydration processes and resulting in increased expansion. The effect of blood contamination on dye leakage of an initial prototype of MTA was investigated in an ex vivo endodontic surgery model, which concluded that blood contamination had no significant effect on dye leakage (Torabinejad et al. 1994). The beneficial reduction in dye leakage (Torabinejad et al. 1994) and bacterial penetration (Montellano et al. 2006) of blood-contaminated samples of MTA might be explained by the expansion of samples when exposed to blood proteins. In addition, Reyes-Carmona et al. (2009) described the formation of an interfacial hydroxyapatite layer with tag-like structures at the junction of MTA and dentine following immersion of MTA samples in PBS, suggesting better adaptation. Shokouhinejad et al. (2010) compared MTA samples exposed to various pH solutions of PBS and described a significantly lower push-out resistance of MTA samples subjected to an acid environment because of reduced adhesion of MTA to dentine. Conversely, VanderWeele et al. (2006) reported blood-contaminated MTA to have a decreased push-out resistance compared to controls, which is not in accordance with the expected expansion or hybrid layer formation in blood-contaminated MTA.

As an incidental SEM finding, incorporation of cotton fibres into the surface of uncontaminated MTA, which was in contact with a moist cotton pellet, was observed (Fig. 6). Therefore, for future studies and clinical applications, the use of an absorbable and nonfibrous material to maintain humidity during MTA setting is advisable.

**Conclusion**

The further blood becomes incorporated into MTA, the more the compressive strength of the material is reduced. At the microstructure level, blood contamination of MTA resulted in a lack of acicular crystals, which can explain the reduction in compressive strength.
strength. Therefore, in clinical situations in which blood becomes incorporated into MTA, its physical properties are likely to be compromised. Future studies are required to determine the full and long-term importance of blood contamination on MTA and its effect on structural porosity and formation of acicular crystals.

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