Physicochemical Basis of the Biologic Properties of Mineral Trioxide Aggregate

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Abstract

This study characterized the interactions of mineral trioxide aggregate with a synthetic tissue fluid composed of a neutral phosphate buffer saline solution and root canal dentin in extracted human teeth using inductively coupled plasma—atomic emission spectroscopy, scanning electron microscopy, energy dispersive X-ray analysis, and X-ray diffraction. Mineral trioxide aggregate exposed to synthetic tissue fluid at 37°C released its metallic constituents and produced precipitates with a composition and structure similar to that of hydroxyapatite [$Ca_{10}(PO4)_6(OH)_2$–HA]. Endodontically prepared teeth filled with mineral trioxide aggregate and stored in synthetic tissue fluid at 37°C for 2 months produced at the dentin wall an adherent interfacial layer that resembled hydroxyapatite in composition. The authors conclude that Ca, the dominant ion released from mineral trioxide aggregate, reacts with phosphates in synthetic tissue fluid, yielding hydroxyapatite. The dentin—mineral trioxide aggregate interfacial layer results from a similar reaction. The sealing ability, biocompatibility, and dentinogenic activity of mineral trioxide aggregate is attributed to these physicochemical reactions.

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Mineral trioxide aggregate (MTA) is a mechanical mixture of three powder ingredients: Portland cement (75%), bismuth oxide (20%), and gypsum (5%) (1). It also contains trace amounts of SiO_2, CaO, MgO, K_2SO_4 and Na_2SO_4. The major component, Portland cement, is a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite. MTA is prepared as a mixture of powder and water and is used in a slurry form, which gradually hardens in the oral environment.

Since its introduction as a root-end filling material in 1993, the use of MTA has expanded to many applications of root repair and bone healing (2–4). These applications include direct pulp capping, repair of root and furcation perforations, and apexification. A material for such applications should have the ability to seal the dental pulp from bacterial and chemical invasion, and the candidate material should be biocompatible to prevent toxicity and tissue irritability. Both in vitro and in vivo studies have shown that MTA fulfills these requirements quite satisfactorily. The superior sealing ability of MTA over conventional retrograde filling materials, such as amalgam, IRM, and Super EBA, has been demonstrated in numerous microleakage tests using dye, fluid, bacteria, and endotoxin infiltration techniques (5–10). Its excellent biocompatibility has been evidenced in several favorable biologic processes induced by MTA, namely, minimal toxicity and pulpal irritation, mild periapical inflammation, nonmutagenicity, cell adherence and growth, increased levels of alkaline phosphatase and osteocalcin, interleukin production (IL-6, IL-8), periodontal ligament attachment, cementum growth, and dentinal bridge formation (11–26).

Studies on MTA have mainly examined its various biologic properties, but little or no attention has been paid to the fundamental physicochemical interaction between MTA and the oral environment that instigates those biologic responses. The purpose of this study was to elucidate the nature of this interaction, specifically to characterize the interaction of MTA with (a) a synthetic tissue fluid (STF) and (b) endodontically prepared root canal walls in extracted human teeth. These environments were chosen to simulate the in vivo conditions in which MTA is used.

Materials and Methods

This study was divided into two parts. In Part I, slurry samples were prepared in six sealable plastic vials using 0.25 g of MTA and 1 mL of distilled water in each vial. Immediately afterwards, 10 ml of an STF was added to each sample, and all samples were stored at 37°C. The STF was a phosphate buffer saline solution (pH = 7.2) of the following composition: 1.7 g KH_2PO_4, 11.8 g Na_2HPO_4, 80.0 g NaCl, and 2.0 g KCl in 10 L of H_2O. Within 1 to 2 hours of storage, white precipitates grew on the surface of samples as well as in the surrounding solutions. After 3 days, the set samples were removed from respective vials in one group of samples (n = 3), and solutions with precipitates were analyzed by inductively coupled plasma—atomic spectroscopy. After 2 weeks, the precipitates from remaining samples were filtered, washed, dried, and characterized by scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDXA), and X-ray diffraction (XRD) techniques. For XRD, a Ni-filtered CuKα radiation was used. In Part II, the canals of two extracted single-rooted human teeth were prepared endodontically with ProFile (Dentsply/Maillefer, Tulsa, OK, USA) sizes 20, 25, 30, and 35, to the apex, with crown-down technique (Sequence Profile O.S./.06/.04), irrigation with 5.25% sodium hypochlorite (NaOCl) to remove smear layer, Glyde File Prep (Dentsply/Maillefer) as chelating agent, and filled with a slurry of MTA prepared in the aforementioned manner. The filled teeth were exposed to the STF at 37°C. After 2 months of exposure, the teeth were sectioned perpendicular to the root canals with a

diamond blade. The apical third sections were then vertically mounted in cold cure epoxy and final polished with 1-μ diamond. The polished cross-sections with exposed MTA-dentin interface were examined by optical microscopy, SEM, and EDXA.

Results

Part I

The concentrations of cations (mean ± SD, ppm) leached from MTA into STF in 3 days were as follows: Ca 176.67 ± 3.30, Si 13.43 ± 0.58, Bi 6.10 ± 0.45, Fe 2.47 ± 0.40, Al 2.27 ± 0.15, and Mg 1.0 ± 0.1.

Scanning electron microscopy of the precipitates collected from the solution revealed that they were globular (Fig. 1A). The individual globules in Fig. 1A appeared to be clusters of numerous minute particles. X-ray analysis indicated that the precipitates contained mainly O, Ca, and P, with trace amounts of Bi, Si, and Al. A typical X-ray spectrum illustrating this analysis and the semiquantitative composition data derived from this spectrum are shown in Fig. 1B. Further XRD analysis (Fig. 1C) indicated that the diffraction lines of the precipitates matched that reported for hydroxyapatite (HA), the main mineral component of teeth and bone (27). SEM examination of the surface of MTA exposed to the STF showed to be covered with precipitates of similar morphology and chemical composition.

Part II

Optical microscopic examination of MTA-dentin cross-sections showed a white interfacial layer sandwiched between MTA and the dentinal wall (Fig. 2A). The layer appeared to be growing inward. Typical SEM micrographs of a section of the interface are shown in Fig. 2, B and C. Because of cracking due to desiccation and a high vacuum of SEM, MTA is separated from the interfacial layer. In spite of this separation, the most notable feature in these figures is that the interfacial layer is firmly attached to the dentinal walls with no observable gaps at the interface.

The elemental composition profiles obtained by X-ray analysis of areas M, I, and D in Fig. 2C are shown in Table 1. The X-ray data from area M, representing MTA, are qualitatively in general agreement with that for MTA. Similarly, the X-ray spectrum from dentin, area D, reflects the composition of its main inorganic component, HA, which contains Ca, P, and O. The composition of the intermediate layer, I, is different from that of MTA in that it shows (a) reduced amounts of Al and Si than are present in MTA and (b), remarkably, the presence of P, which is not a component of MTA. One should note that this area was occupied solely by MTA before its exposure to the STF. Apparently, its composition had changed during its 2-month exposure to the simulated oral environment.

Discussion

The inductively coupled plasma—atomic spectroscopy data presented above indicate that MTA undergoes dissolution in STF, releasing all of its major cationic constituents. The additional cation Mg found in STF is believed to have its origin in MgO, present in MTA in trace amounts. Of all ions released, Ca is the most dominant. Because it is sparingly soluble in biologic fluids, it leads to the precipitation of HA (Fig. 1A). The specific reaction responsible for this precipitation may be the following:

$$10\ Ca^{+2} + 6(PO_4)^{-3} + 2(OH)^{-1} \rightarrow Ca_{10}(PO_4)_6(OH)_2$$

The above is a well-known reaction in the biologic calcification process (27–28) and is favored at pH = 7, the pH of STF used in this study. This reaction occurs in vivo and in vitro with many Ca-containing materials in contact with biologic environments (29–34). An essentially similar reaction, we believe, is also responsible for the formation of the adherent HA layer on the MTA surface (Fig. 2, A–C). Because of the porous nature of MTA, it is conceivable that this precipitation continues internally within MTA and thus leads to a change in the overall composition of MTA adjacent to the dentinal wall (Table 1).

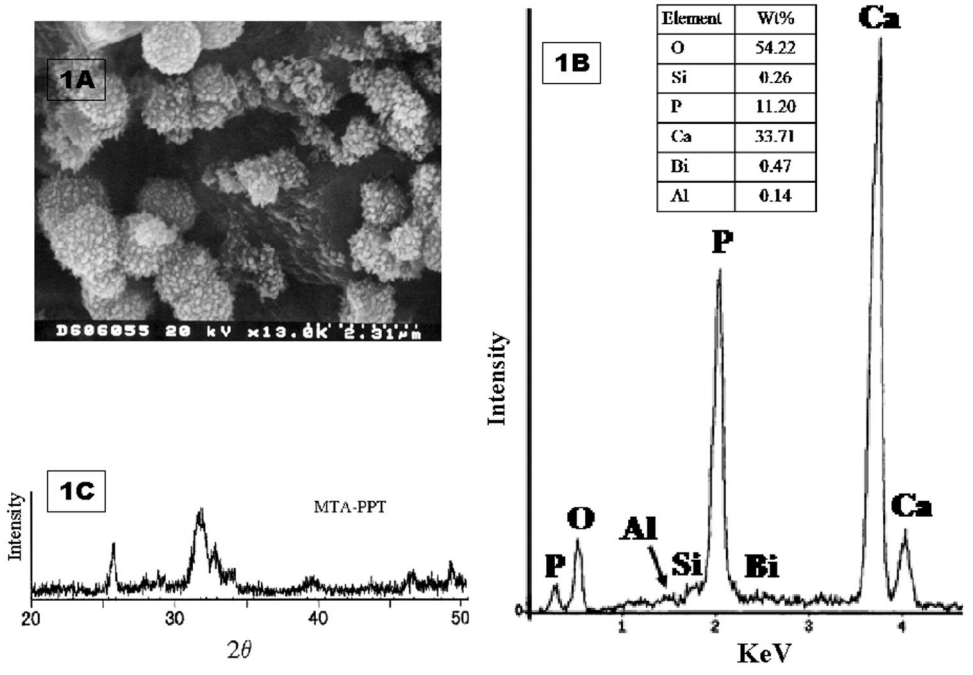

Fig 1. **A,** Typical scanning electron micrograph of precipitates from mineral trioxide aggregate—synthetic tissue fluid interaction (×13,000). **B,** Energy dispersive X-ray analysis spectrum from precipitates in A (above) and their semiquantitative chemical composition (below). **C,** X-ray diffraction pattern of mineral trioxide aggregate—synthetic tissue fluid precipitates.

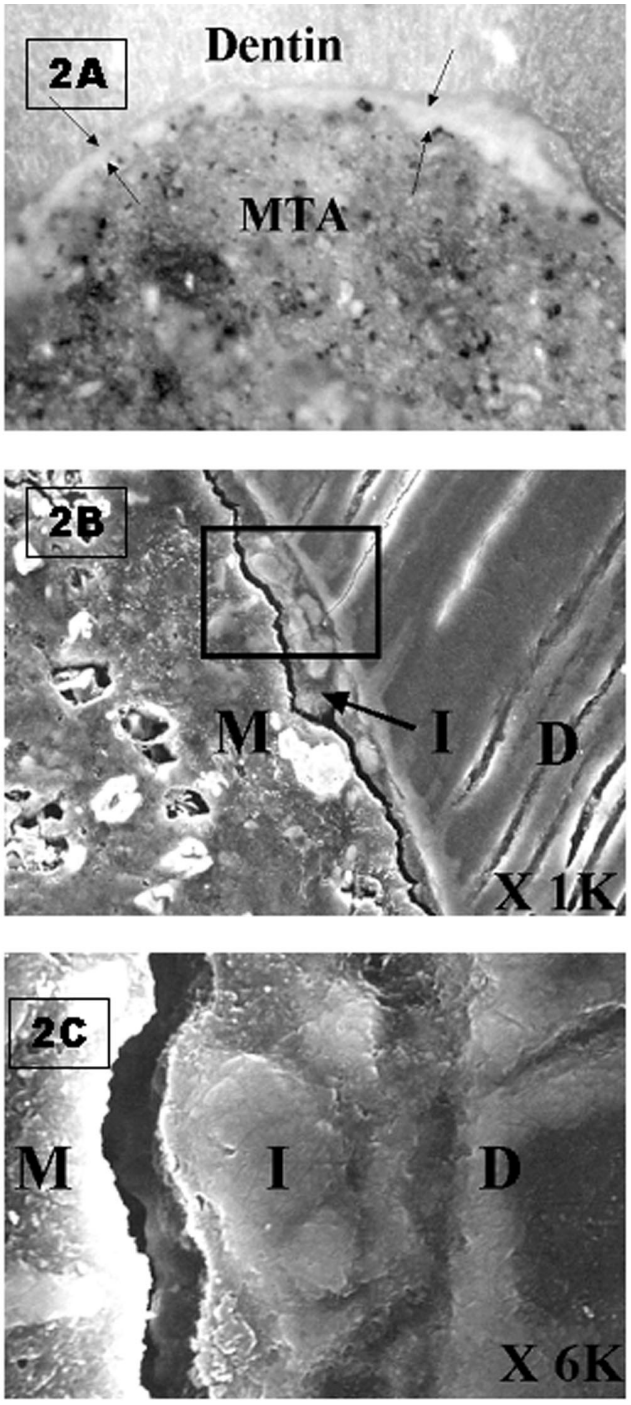

Fig 2. **A,** Typical optical micrograph of a mineral trioxide aggregate—dentin cross-section (×200). **B,** Typical scanning electron micrograph of a mineral trioxide aggregate (MTA)—dentin cross-section (×1000). M, MTA; I, interface; D, dentin. **C,** Area identified by box in B at a higher magnification: ×6000.

A material with an apatitic layer on its surface that is in proximity to calcified tissues forms a chemical bond with the latter (29–32). Because on exposure to STF, MTA is covered with a layer of apatite, the firm attachment of dentin to MTA appears to reflect such a chemical bond.

Thus, we suggest that the two significant properties of MTA, namely, sealing ability and biocompatibility, emanate from the physicochemical reactions discussed above. To clarify, it can be envisioned that after the placement of MTA in root canals and its gradual dissolution, HA crystals nucleate and grow, filling the microscopic space between MTA and the dentinal wall. Initially, this seal is mechanical. With time, we conjecture that a diffusion-controlled reaction between the apatite layer and dentin leads to their chemical bonding. The result is the creation of a seal at the MTA-dentine interface.

It is interesting that the distinct interfacial layer (Fig. 2, A–C) observed in the present study is comparable to the hard-tissue layer that forms in apposition to MTA in biologic environments. Histologically, this layer has been described as dentinal bridge, osteotypic matrix, osteodentin, and reparative dentin in various animal and human studies (18, 20, 22–24). Because of its birefringent characteristic under polarized light, this structure has been suggested to be calcite, a compound of calcium and carbonate (20). The tissue fluid is highly rich in phosphate ions; its carbonate content is relatively low (28). In such a milieu, chemically the formation of HA is more favorable than calcite (27–28). Our interpretation is strongly supported by the results of a recent study on the dentinogenic activity of MTA in dog pulps (23). This study showed the growth of crystalline deposits on the surface of MTA and a zone of crystalline structures along the pulp-MTA interface. One of the crystalline structures analyzed was found to contain Ca and P, suggesting the formation of HA. In another study, dentinal bridges enriched with Ca and P were identified in dog pulps after pulp capping and pulpotomy treatments with MTA (24). These observations, together with our data and the information on the biologic calcification process (27–28), lead us to surmise that the product of the reaction of MTA with the oral environment is not calcite but HA.

Hydroxyapatite itself and some other Ca-containing materials exhibit excellent biocompatibility manifested in minimal tissue toxicity and foreign-body reaction, osteoinductivity, and osteogenicity (27, 29–32). The reason for this characteristic may be their ability to release calcium and phosphate ions, which are critical factors in bone metabolism. Because MTA releases Ca ions and contributes to the formation of HA in synthetic biologic fluids, its favorable biologic properties are thus not surprising. In this context, it is worth noting that several materials, namely $Ca(OH)_2$, calcium phosphate cements, hydroxyapatite cement, and Portland cement elicit biologic responses essentially similar to that of MTA (33–37). It appears from the preceding discussion that this similarity in their mode of biologic action stems from one common characteristic they all possess: their propensity to release Ca and ability to form HA.

We conclude that MTA is not an inert material in a simulated oral environment; it is bioactive. In contact with an STF, it dissolves, releasing all of its major cationic components and triggering the precipitation of HA on its surface and in the surrounding fluid. It appears to bond chemically to dentin when placed against it, possibly via a diffusion-controlled reaction between its apatitic surface and dentin. The clinical success of MTA, in terms of its sealability, biocompatibility, and denti-

TABLE 1. Semi quantitative elemental composition (wt%) of areas identified as M, I and D in Fig. 2C.

Area	Ca	Al	Si	Bi	Fe	Mg	O	S	C	P
MTA	21.1	2.6	11.8	7.8	7.5	1.4	41.5	1.3	5.0	–
Interfacial layer	21.5	0.6	3.0	5.6	–	0.1	60.6	–	4.9	3.7
Dentin	31.7	–	–	–	–	0.4	50.8	–	6.0	11.1

nogenic activity, we believe, is rooted in the aforementioned physicochemical reactions.

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