▶ ORIGINAL ARTICLE ◀

Interfacial layer between root dentin and mineral trioxide aggregate mixed with phosphate-buffered saline solution in a root-canal model: A preliminary study

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Abstract

Objective: To investigate the effect of mixing phosphate-buffered saline (PBS) solution with mineral trioxide aggregate (MTA) on interfacial-layer formation between MTA orthograde filling and root dentin in a root-canal model using scanning electron microscopy (SEM) and energy-dispersive x-ray spectroscopy (EDXS).

Materials and methods: Each of the four human mandibular premolars was horizontally sectioned to obtain a 12-mm length of the root. The root canal was sequentially prepared by a series of peeso-reamers (sizes 2–4) to simulate a root-canal specimen of the immature tooth with an opened apex. The two specimens were filled with MTA (*ProRoot MTA*) mixed with PBS solution (MTA/PBS) while the remainder were filled with MTA mixed with distilled water (MTA/DTW). MTA was packed into the canal for an 8 mm length, and the 4-mm remaining canal was filled with small moist cotton and a temporary restorative material. All specimens were stored in PBS solution for 4 weeks. For the experimental specimens of MTA/PBS, the roots were sectioned at the coronal, middle, and apical levels of MTA whereas the roots of MTA/DTW (control) were sectioned at the middle level. The specimens were prepared for SEM and EDXS examination. Representative interfaces and EDXS analysis were investigated at 3,000x magnification under SEM.

Results: SEM images and EDXS graphs of MTA/DTW specimens showed no phosphate-containing interfacial layer was formed. SEM images and EDXS graphs of MTA/PBS specimens showed a formation of the interfacial layer containing the phosphate element at all root canal levels. At the coronal and middle levels, the peaks of phosphate in the interfacial layer were lower than those in root dentin. At the apical level, the peak of phosphate in the interfacial layer was as high as that in the dentin.

Conclusion: MTA mixing with PBS solution tends to induce an interfacial layer formed between MTA material and root dentin, particularly at the apical level.

Keywords: dentin, interfacial layer, mineral trioxide aggregate, phosphate-buffered saline, root canal

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Introduction

Mineral trioxide aggregate (MTA), a calcium silicate-based material, was developed and first introduced in 1993 (1). This bioactive material can be used in endodontic applications such as pulp capping, root-end filling, root perforation repair, or orthograde apical barrier (apexification) (2). Original MTA powder is mainly composed of tricalcium silicate and dicalcium silicate in combination with a radiopacifier (i.e. 20% bismuth oxide) (1). The MTA powder is mixed with a liquid (i.e. distilled water) in a powder-liquid ratio of 3:1. The hydration reaction after mixing produces calcium aluminate hydrates, calcium silicate hydrates, and calcium hydroxide (3). MTA requires a setting time of approximately 4 h (1, 4). The high alkaline pH of MTA is from the release of calcium ions that is responsible for antibacterial activity and bio-mineralization induction (1, 5, 6). Moreover, 3-4 mm thick MTA provides an excellent sealing ability that prevents bacterial recontamination into the root canals (7, 8). MTA sealing is explained by the expansion of material after setting (9, 10) as well as the bioactive induction of apatite formation in the interfacial layer between the material and root dentin after immersion in a phosphate-containing solution (11).

The bioactive nature of MTA has been proved in many *in vitro* studies using a material-disc model (6, 11-15). Sarkar, et al. (16) found globular precipitates, which their compositions are similar to hydroxyapatite (HA), on the surface of MTA after immersion in phosphate-buffered saline (PBS) solution for 2 weeks. Other studies confirmed that the precipitates possess physical and chemical structures similar to HA (17-20). HA-like precipitates rapidly form within the first few hours after immersion of MTA in the phosphate-containing fluid. The precipitation on the surface of MTA increasingly develops, and the precipitates entirely cover the material surface after immersion for 7 days (6). Calcium ions are the major element released from MTA that reacts with the phosphate-containing fluid to induce the formation of these crystalline precipitates (16, 17, 19, 20). For other solutions such as distilled water, the precipitates on the material surface are not hydroxyapatite, but calcium hydroxide is formed instead (20).

The formation of an interfacial layer from the interaction between MTA and root dentin has been reported in a root-canal model (12, 13). In root canals filled with MTA and immersed in PBS solution for a period, the interfacial layer is observed at the cement-dentin interface that has contacted the solution. In addition, tag-like interfacial structures are detected in dentinal tubules. Kim, et al. (15) reported that this interfacial layer has a chemical composition and physical structure similar to HA. This reaction zone possesses the phosphate component (16). The calcium/phosphate (Ca/P) ratio of the interfacial layer ranges between 5.1 to 5.8 (12, 15, 16). However, this Ca/P ratio is much higher than that of the crystalline hydroxyapatite with a Ca/P ratio of 1.67 or other calcium phosphate compounds (e.g. tricalcium phosphate with a ratio of 1.5) (21). The type of enriched calcium-phosphate precipitates in the interfacial layer is still unclear.

Biomineralization of MTA by induction of HA-like precipitate formation was commonly observed in a dentin-disc model with immersion in the PBS solution (6, 12). However, MTA orthograde filling in the root canal (e.g. apexification or apical barrier material) has no direct contact with tissue

fluid from the periapical area except the MTA surface at the apical end. Reyes-Carmona, et al. (13) investigated the bioactivity of MTA apical plug in a root-canal model and found that the interfacial mineralization zone was formed only on the apical surface after immersion in the PBS solution for 2 months. Therefore, the induction of an interfacial mineralization layer between MTA and dentin inside the root canal is challenging since any diffusion of tissue fluid into the filled canal is not anticipated.

MTA orthograde filling in the root canal may bond to root dentin by forming an interfacial HA-like layer. However, the phosphate-containing solution is required for the chemical interaction, and the material must be immersed in the solution for several weeks (6, 12). From a laboratory study using a root-canal model, intracanal dressing with PBS solution over the MTA orthograde filling for 2 months triggers the formation of the interfacial layer, particularly in the coronal part of the material (13). However, in clinical practice, root canals are commonly irrigated with sodium hypochlorite, ethylene diamine tetra-acetic acid, and/or chlorhexidine solutions before MTA apexification. In addition, at the next visit (usually within a week), the remainder of the root canal is then filled with gutta-percha and sealer after MTA is set. Without the long-term PBS intracanal dressing, HA-like precipitates may not form inside the root canal after MTA orthograde filling even though precipitation formation is anticipated at the apical end with the presence of the periapical tissue fluid (13). PBS solution may be used as a final irrigant to induce the interaction between MTA and root dentin and the formation of an interfacial layer. Nevertheless, the irrigation time and the amount of phosphate-containing solution remaining on root dentin are much less than those by immersion in the PBS solution. Hence, the effect of PBS irrigant before MTA orthograde filling on interfacial-layer formation seems to be very limited (13). Mixing PBS with MTA may be an alternative for the induction of the interfacial layer by the phosphate solution inside the mixed material (22). Filling root canals with MTA mixed with PBS solution increased the fracture resistance of the roots compared to those filled with MTA mixed with distilled water (22). However, the interfacial layer formation between MTA mixed with PBS and root dentin has not been previously investigated.

Therefore, the objective of this preliminary study was to investigate the effect of PBS solution mixing with MTA on interfacial layer formation between MTA orthograde filling and root dentin in a root-canal model using scanning electron microscopy (SEM) and energy-dispersive x-ray spectroscopy (EDXS).

Materials and methods

In this preliminary study, four human single-root-canal mandibular premolars were randomly selected from a pool of anonymous extracted teeth in a container immersed in 0.1% thymol solution. The root tip of each tooth approximately 3 mm in length was sectioned and discarded. The coronal end of the root was horizontally sectioned to obtain a 12-mm length of the root. Pulpal remnants were removed using a hand file size 10–15 (*Dentsply Maillefer, Tulsa, OK, USA*). The root canal was initially prepared by the hand files until the size 40 and then sequentially prepared using a series of peeso-reamers (*Dentsply Maillefer*) sizes 2–4 to simulate a root-canal model of the immature tooth with an opened apex. The root canal was irrigated with 5 ml of 2.5% sodium hypochlorite (NaOCl), 3 ml

of 17% ethylenediamine tetra-acetic acid (EDTA), and then 5 ml of 2.5% NaOCl. The root canal was finally flushed with PBS solution (*Sigma-Aldrich, Inc., St. Louis, MO, USA*) (concentration of 137 mM NaCl, 2.7 mM KCl, and 10 mM phosphate buffer solution; pH 7.4 at 25 °C) for 5 ml and dried with paper points.

The two root-canal specimens were filled with MTA (*ProRoot MTA, Dentsply Sirona, Charlotte, NC, USA*) mixed with the PBS solution (MTA/PBS) while the other two specimens were filled with MTA mixed with distilled water (MTA/DTW) (served as a control). The powder-liquid mixing ratio was 3:1 (by weight) in both groups. MTA was mixed with the liquid on a mixing pad, loaded into the root canal using an MTA carrier (*Dentsply Maillefer*), and plugged with an endodontic plugger. The sectioned root was placed and fixed on a glass slide to prevent the extrusion of the MTA material. The MTA was packed into the canal for an 8 mm length, and the 4-mm remaining canal was filled with a thin moist cotton pellet and a temporary restorative material (Cavit, *GC Corp., Banksmeadow, NSW, Australia*). For both groups, the MTA material was left for an initial set for 4 h before the specimen was immersed in 40 ml PBS solution in a container for 4 weeks, during which the PBS solution was renewed every week.

After 4 weeks, the root specimens were removed from the solution. For the experimental specimens of MTA/PBS, the roots were sectioned at the three levels– coronal, middle, and apical (2, 4, and 6 mm from the uppermost level of MTA) using a carborundum disc in a low-speed handpiece. The roots were only sectioned at the middle level for the controls (MTA/DTW). The sectioned specimens were sequentially polished with silicon carbide papers grit 600–4,000 (10 times each) before ultrasonic cleaning in distilled water for 5 min. The surfaces of sectioned specimens were treated with 17% EDTA for 5 sec and 1.25% NaOCl for 5 min for smear layer (from the cutting/polishing process) removal. The specimens were immersed and dehydrated in a serial dilution of ethyl alcohol (20 min in 20 ml of each dilution of ethyl alcohol from 50–100%). The specimens were dried and coated with palladium before SEM and EDXS examination.

The interfaces between MTA/PBS (or MTA/DTW) and the root dentin were initially investigated around the root canal walls at low (15x) and medium (1,000x) magnifications. The microscopic details and EDXS analysis of representative interfaces were investigated at high (3,000x) magnification under SEM (*JSM-6610LV, JEOL Ltd., Tokyo, Japan*). A graph of EDXS analysis in the representative area of each specimen was plotted by removal of the palladium (used for coating) peak. The results were descriptively analyzed and explained.

Results

The results of SEM and EDXS are shown in Figures 1–4, in which the results of the two specimens in each group were similar. A representative specimen of MTA/DTW was explored at the mid-root level to observe the interface between the MTA orthograde filling and the root dentin under SEM at different magnifications (Fig. 1A-1C). SEM images at 3,000x magnification and relating EDXS graphs of a representative specimen in the control group (MTA/DTW) were presented in Figure 1D-1I. The SEM images and EDXS graphs showed that the MTA material attached to the root dentin, but none of the phosphate-containing interfacial layer was formed (Fig. 1D-1G)– a peak of phosphate in the EDXS graph was only found in the dentin (Fig. 1H-1I).

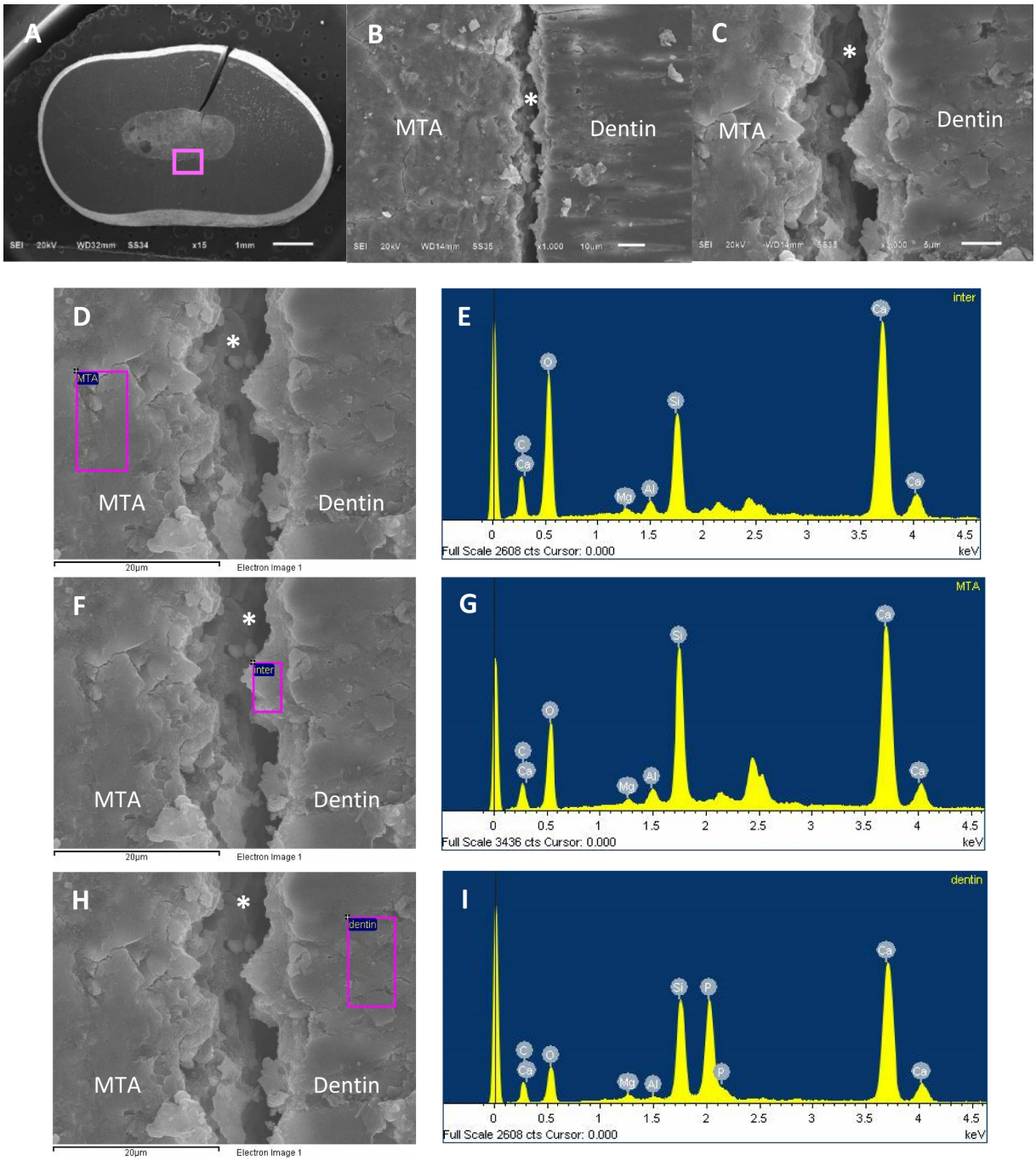

Figure 1: SEM/EDXS analysis of MTA orthograde filling (mixed with distilled water– control) in a root canal sectioned at the middle level of the root after PBS solution was used as a final irrigant. A–C: SEM images (15x, 1,000x, and 3,000x magnifications) show the MTA-filled root canal and the representative interfaces between MTA and root dentin (the rectangular area in Fig.1A). D–I: SEM images and relating EDXS graphs in the areas of MTA, interface, and dentin (the rectangles), respectively. The peak of phosphate (P) was only detected in the dentin (Fig.1H-I); none of the peaks was found in MTA (Fig.1D-E) or the interface (Fig.1F-G) (*the artificial gap from the drying process in specimen preparation before SEM investigation).

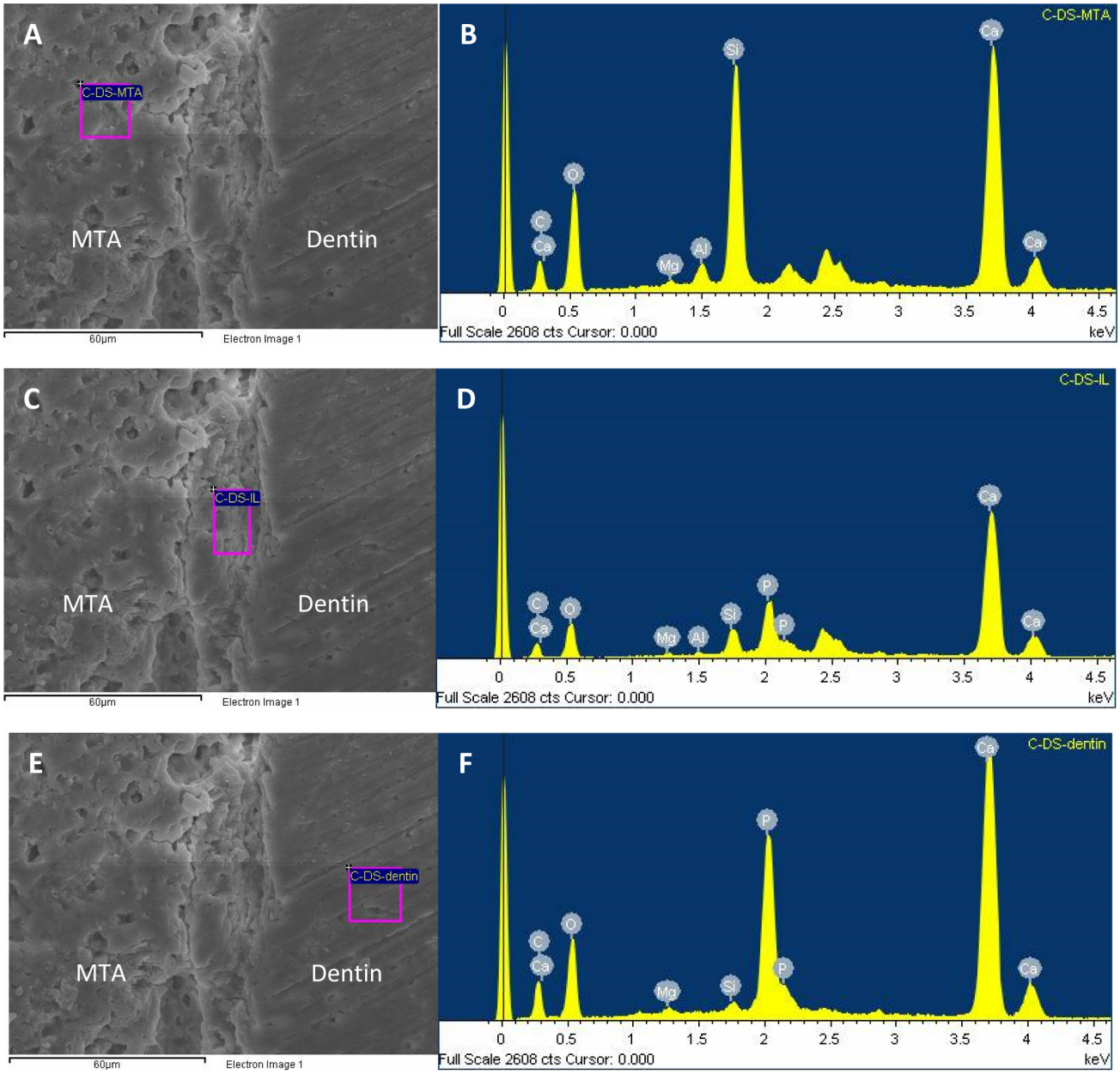

Figure 2: SEM/EDXS analysis of MTA mixed with PBS solution in a powder-liquid ratio of 3:1 (horizontal section– the coronal level). A–F: SEM images and EDXS graphs in the areas of MTA, interface, and dentin (the rectangles), respectively. The phosphate (P) peak was detected in the interfacial layer (Fig. 2C-D), but the height of the P peak was lower than that of the dentin (Fig. 2E-F). The P peak was not detected in the MTA (Fig. 2A-B).

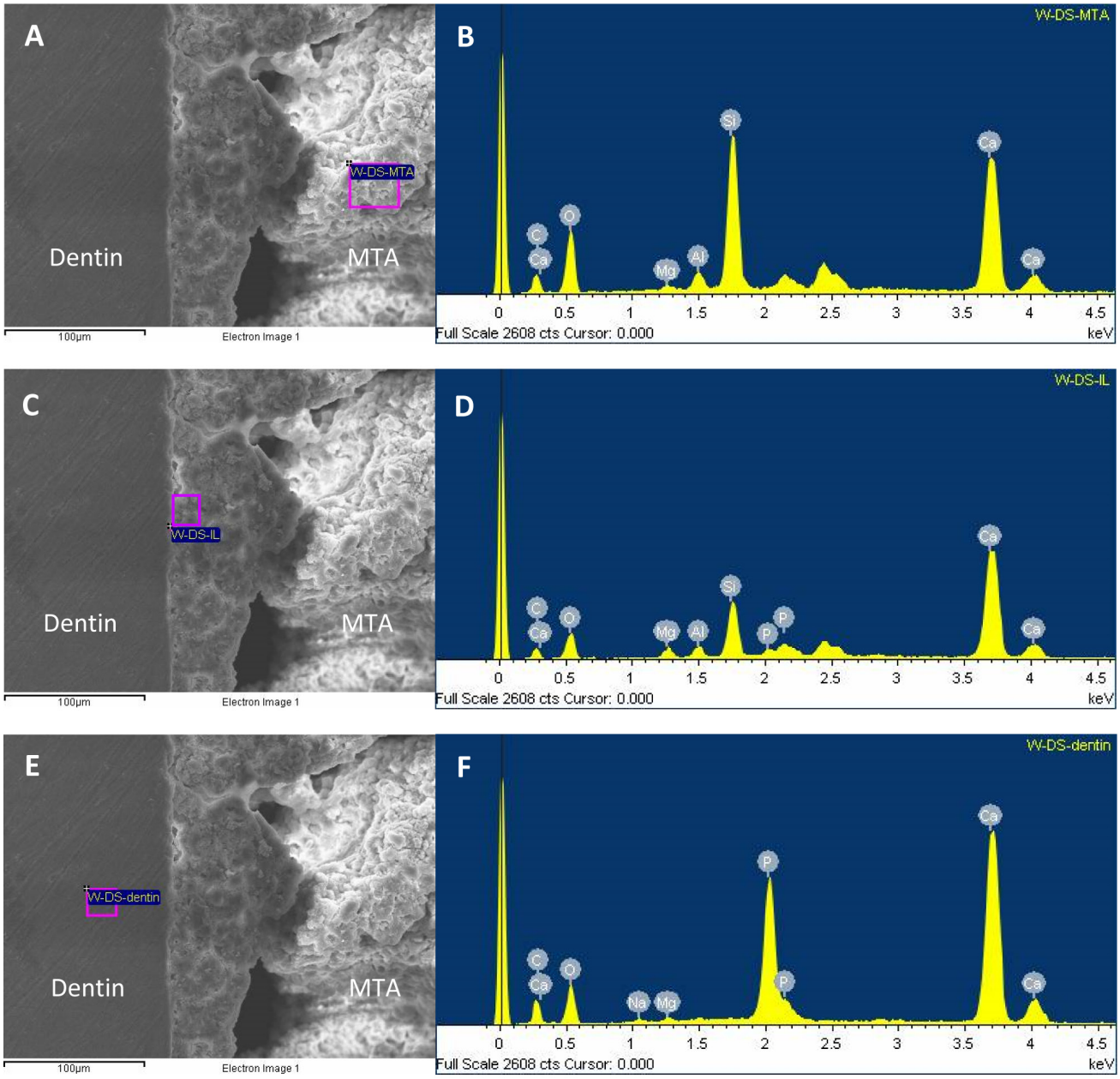

Figure 3: SEM/EDXS analysis of MTA mixed with PBS solution in a powder-liquid ratio of 3:1 (horizontal section– the middle level). A–F: SEM images and EDXS graphs in the areas of MTA, interface, and dentin (the rectangles), respectively. The peak of phosphate (P) was detected in the interfacial layer (Fig. 3C-D), but the height of the P peak was markedly lower than that found in the dentin (Fig. 3E-F). The P peak was not detected in the MTA (Fig. 3A-B). The artificial gap was observed between the MTA and the interfacial layer.

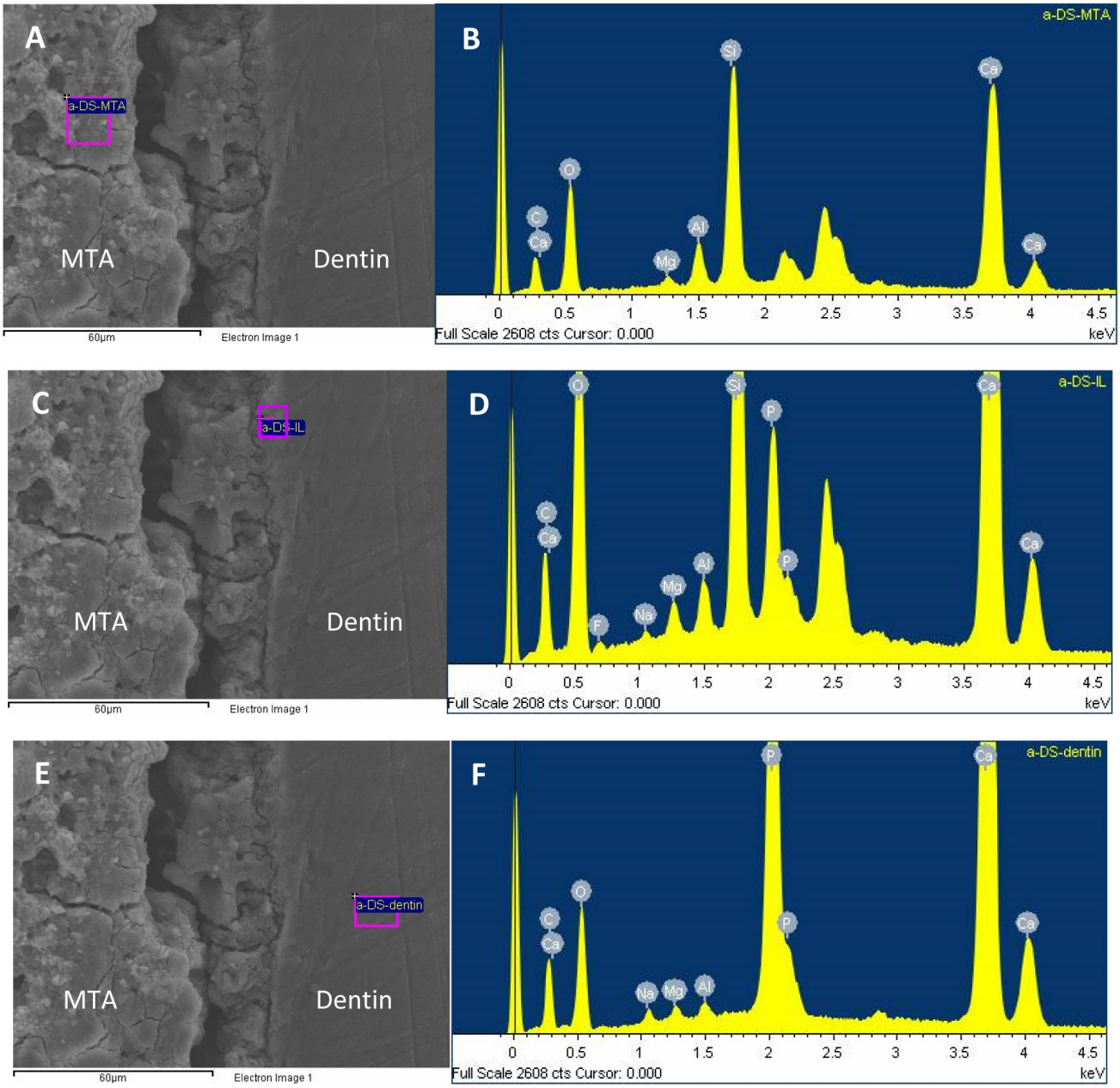

Figure 4: SEM/EDXS analysis of MTA mixed with PBS solution in a powder-liquid ratio of 3:1 (horizontal section– the apical level). A–F: SEM images and EDXS graphs in the areas of MTA, interface, and dentin (the rectangles), respectively. The peak of phosphate (P) was detected in the interfacial layer (Fig. 4C-D) with the P peak being as high as that detected in the dentin (Fig. 4E-F). The P peak was not observed in the MTA (Fig. 3A-B). The artificial gap was observed between the MTA and the interfacial layer.

In contrast, SEM images and EDXS graphs from a representative specimen of MTA/PBS showed a formation of the interfacial layer containing the phosphate elements between the MTA mixed with PBS and the root dentin at all levels of root canal- coronal, middle, and apical (Fig. 2–4, respectively). This interfacial layer showed a physical structure different from the MTA material and dentin. At the coronal and middle levels (Fig. 2A–F and 3A–F), the peaks of phosphate in the interfacial layer (Fig. 2 and 3C–D) were lower than those in the dentin (Fig. 2 and 3E–F), and the peaks of silica in the interfacial layer (Fig. 2 and 3C–D) were lower than those in the MTA (Fig. 2A–B). Conversely, at the apical level (Fig.4A–F), the peaks of phosphate and silica of the interfacial layer (Fig. 4C–D) were high and similar to those in the root dentin (Fig. 4E–F) and the MTA (Fig. 4A–B).

Discussion

Our preliminary results showed that MTA mixed with distilled water (as in a marketed product) did not form an interfacial layer inside the root canals (in the areas that did not contact the PBS), which corresponds to the results of other studies (13, 22). Using SEM and EDXS analysis confirmed the topographical structure and the elements, which showed an absence of the interfacial layer. Hence, we decided to only section the specimens in the control group in the middle. By SEM and element analysis, most of the laboratory studies could induce the HA-like precipitates from MTA by immersion of the specimen in a simulated body-fluid, phosphate-containing solution (6, 12, 15, 17, 19, 23). Hence, many attempts have been tested to induce the precipitating interfacial layer formation in root canals. For example, PBS intracanal dressing for 8 weeks could induce interfacial layer formation (13), though this method was not practical. However, these 8 weeks was used for immersion of the specimens in our study.

Mixing PBS solution with MTA is another attempt to induce the formation of HA-like precipitates. Our SEM and EDXS results showed the formation of the interfacial layer in topographical and elemental features for MTA/PBS. The interfacial layer may comprise of amorphous calcium phosphate or HA-like structure (6, 11, 13, 15-17). No other study has previously investigated whether mixing with PBS may enhance the bioactivity of MTA. A laboratory study reported that MTA/PBS improved the push-out bond strength in the root canals compared to MTA/DTW, which may be explained by biomineralization induction in MTA/PBS (24). However, the formation of the biomineralization interfacial layer has not been confirmed in that study. The MTA zone did not show the phosphate peak in the EDXS graph even though the material was mixed with phosphate-containing PBS. This may be expected that a very low amount of phosphate remained in the material layer, which the EDXS could not detect.

The interfacial layer in the apical level of the root canal showed a peak of phosphate as high as that of root dentin. Moreover, the phosphate peak of the apical level was much higher than those of the coronal and middle levels. The specific characteristic of the apical interfacial layer may be explained by the storage by immersion of the specimen in the PBS solution in our study, in which direct contact between the material and PBS could

enhance the phosphate-related biomineralization (12). In addition, diffusion of PBS into the MTA material may also improve the bioactivity and the amount of phosphate in the interfacial layer (13). *In vivo*, the formation of a high-phosphate interfacial layer is possible, in which the material at the apical level contacts (directly and/or indirectly) with the tissue fluid containing the phosphate in the periapical area.

The physical and mechanical properties of MTA mixed with PBS are a concern. Mixing PBS into MTA may delay the setting reaction and decrease the release of calcium hydroxide (25). Therefore, other properties of MTA/PBS should be further tested. Moreover, if the properties of MTA are inferior by mixing with PBS, an investigation in a proper ratio of PBS and distilled water for mixing with MTA powder will be interesting (e.g. mixing with 25–50% PBS, not 100% PBS as did in this study). It would be great if a balance between bio-induction and physical/mechanical properties is possible. In addition, the premolars used in this study were probably collected from young patients (anonymous specimens) due to non-attrition and non-abrasion tooth anatomy. The results of the elderly teeth may be different and should be further tested. For the immersion solution, PBS was not as same as the tissue fluid, so a simulated body fluid or serum should be tried. Finally, this preliminary study is just the beginning to confirm the possibility of using PBS in increasing MTA bioactivity. Further *in vitro* and *in vivo* investigations are required in the development of a PBS-mixed MTA material.

Conclusion

Within the limitations of this preliminary study in the root-canal model, MTA mixing with PBS solution tends to induce an interfacial layer formed between the MTA material and the root dentin, particularly at the apical level.

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Conflict of interest

None.

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