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Calcium ion diffusion from mineral trioxide aggregate through simulated root resorption defects

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Abstract – The purpose of this study was to investigate the diffusion of calcium ions (Ca^{+2}) through exposed dentinal tubules following intracanal application of mineral trioxide aggregate (MTA). Fifty-two single-rooted teeth were instrumented using 2.5% sodium hypochlorite for irrigation between each file size. Thereafter, standardized defects were created on the root surfaces so as to mimic external root resorption. The root canals and external defects received a final irrigation of 17% ethylenediaminetetraacetic acid and distilled water. MTA powder was then mixed with saline and placed into the canals. All root surfaces except the cavities were sealed with two coats of varnish. Teeth with unfilled canals ($n = 26$) served as controls. The teeth were immersed in saline after which the release of Ca^{+2} from the defects into the saline was measured at 1, 3, 7, 14, and 28 days. The results showed diffusion of Ca^{+2} through the defects in the dentin in MTA-filled roots with a significant increase in concentration within time.

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Mineral trioxide aggregate (MTA) has been a biomaterial of considerable clinical and laboratory research. Besides its potential use as a root-end filling material or a root canal filling material, the use of MTA has been advocated as a repair material for iatrogenic root perforations and several other defects caused by caries, resorption, and trauma (1–5). Among these, treatment of inflammatory root resorption requires elimination of the inflammatory process and inhibition of the activity and formation of resorbing cells (6, 7). To date, the proposed treatment regimens for internal or external root resorption has been limited to the use of using calcium hydroxide or other biomaterials that help promote deposition of a hard tissue barrier, while providing a biologic seal (8).

A large number of studies have shown that MTA is biocompatible, non-toxic, insoluble in the presence of tissue fluids, and is capable of promoting a suitable environment for the regeneration of periradicular tissues (1, 5, 9–11). Moreover, Koh et al. (11) showed that MTA stimulates the regulation of interleukins which are involved in bone turnover. MTA has two specific phases, comprising calcium oxide and calcium phosphate. Calcium oxide reacts with tissue fluids to form calcium hydroxide (1, 10, 12). Fridland and Rosado (13) reported that calcium (in its 'hydroxide' form) is the main chemical compound released by MTA in water. As the main reason for the use of calcium hydroxide-containing medications is to benefit from the diffusion of Ca^{+2} and OH^- ions through dentinal tubules to the root surface (14), release of Ca^{+2} from MTA could yield a desirable healing effect.

Mineral trioxide aggregate has also been suggested for use as a root canal filling material (2, 15). Holland et al. (4) reported that MTA has the ability to stimulate hard tissue deposition at the apical level after root canal filling. This finding could be extended to other levels of the root, with special regard to inflammatory resorption where the remaining dentin could serve as a slow-release system of intracanal MTA-derived Ca^{+2} to the potential healing site. Consequently, the purpose of this study was to determine diffusion of calcium ions through dentinal tubules after intracanal MTA application.

Materials and methods

Fifty-two single-rooted human teeth were extracted, cleaned of soft debris and soft tissue remnants, and stored in physiologic saline at $+4°C$ for a maximum of

1 month. The teeth were transferred to room temperature 24 h before experimental procedures. First, the crowns were removed at the cementoenamel junction using a low-speed diamond disk under coolant water spray. The root canals were instrumented with K-files to size 60 at the established working lengths and, thereafter, with Gates Glidden burs (nos 3, 4 and 5) at the coronal one-third. During cleaning and shaping, irrigation was made with 5 ml 2.5% sodium hypochlorite (NaOCl) at each file and bur size. To simulate external root resorption, standardized defects (3 mm in diameter and 1 mm in depth) were created using an ISO No. 12 cylindrical diamond bur at high speed and water spray, exposing dentin in approximately the middle-third of root surfaces. To determine the remaining dentin thickness after instrumentation, digital radiographs of the specimens were obtained with a parallel technique (at 70 kVp and 0.1 s exposure) using a digital radiography unit (IRIX 70; Trophy, Croissy-Beaubourg, France). Accordingly, the specimens were placed in a custom-made precision platform mounted on the cone of the x-ray tube which enabled standardized alignment of the sensor and the radiographic beam. The digital radiographs were obtained, transferred to a Macintosh G5 PowerPC, and opened in ImageJ open-source image analysis software (V.1.34; National Institutes of Health). For each specimen, three measurements (coronal, middle, and apical levels) were made between the base of the cavity and the canal outline (Fig. 1). The reference for exact calibration of image analysis software was provided by a 2-mm stainless steel orthodontic wire fixed to the platform and radiographed with all teeth.

Following radiographic assessments, the root canals and the external defects were irrigated with 17% EDTA and 2.5% NaOCl (5 ml each) to remove the smear layer and, thereafter, rinsed with 10 ml unbuffered isotonic saline. The root canals were dried with paper points.

The powder of MTA (Pro-Root^{TM} MTA, Dentsply; Tulsa Dental, Tulsa, OK, USA) was mixed with saline in accordance with the manufacturer's recommendations, and the mixture was applied to the canal using a low-speed lentulo spiral until the material reached the canal orifice. The MTA mixture was further condensed with hand pluggers so as to facilitate better adaptation of the material to root canal walls. Finally, the coronal access was sealed with Intermediate Restorative Material (IRM). Half of the specimens ($n = 26$) were left unfilled to serve as controls. The entire root surfaces, except the external defects, were then coated with two layers of nail varnish. After self-drying of the varnish, each sample was immersed in a separate plastic vial containing 10 ml physiologic saline, and transferred to an incubator at 37°C and absolute humidity. The Ca^{+2} concentrations and pH values of the immersion media were measured using an AVL 988-4 analyzer with a calcium ion-selective electrode (AVL Corp., Graz, Austria) at 1, 3, 7, 14, and 28 days. The results were analyzed statistically by one-way analysis of variance with repeated measures at a significance level of $P = 0.05$.

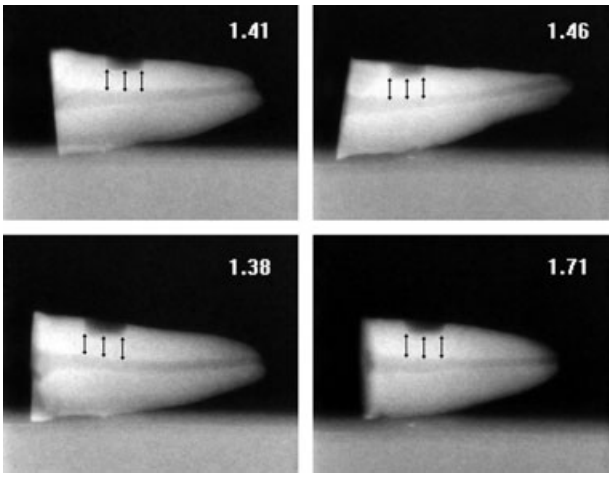

Fig. 1. Typical examples depicting measurement of remaining dentin thickness. Values for each sample (mm) represent the mean value of three measurements. The mean remaining dentin thickness of the specimens was 1.48 ± 0.13 mm.

Results

The changes in pH and calcium ion release within the immersion medium are presented in Tables 1 and 2, respectively. The mean remaining dentin thickness of the specimens was 1.48 ± 0.13 mm. For all samples, the pH values of the immersion media were approximately between 7.40 and 7.50 and did not demonstrate any significant change throughout the test period (Fig. 2, $P > 0.05$). Additionally, there was no significant difference between the pH values of the control and experimental samples at any evaluation period (Table 1, $P > 0.05$).

Initially, (0–3 days), intracanal application of MTA did not cause a significant shift in the Ca^{+2} concentration. After day 3, however, a significant increase was observed in the immersion media (Fig. 3, $P < 0.05$). The control specimens showed a slight initial increase in the

Table 1. Changes in pH within time

	MTA		Control	
Time (days)	Mean	SEM	Mean	SEM
1	7.42	0.008	7.41	0.00
3	7.41	0.012	7.38	0.00
7	7.42	0.010	7.37	0.00
14	7.48	0.09	7.41	0.00
28	7.49	0.06	7.47	0.00

Table 2. Release of calcium ions within time (values are expressed as mg dl^{-1})

	MTA		Control	
Time (days)	Mean	SEM	Mean	SEM
1	0.07	0.06	0.00	0.00
3	0.19	0.02	0.00	0.00
7	0.49	0.06	0.17	0.00
14	1.39	0.22	0.21	0.00
28	3.06	0.31	0.77	0.00

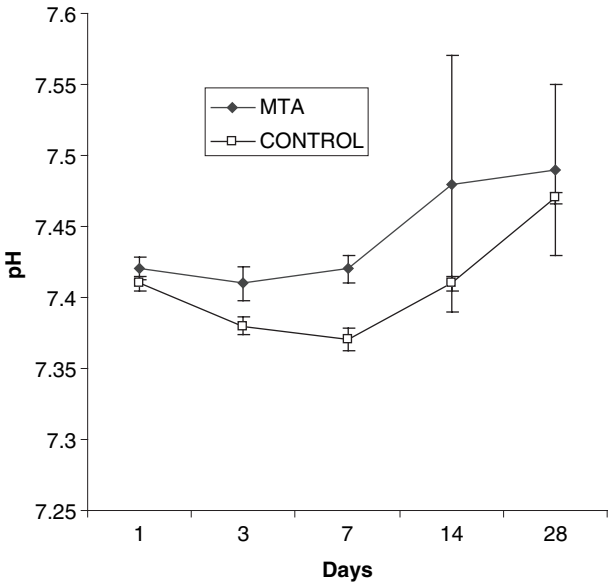

Fig. 2. pH changes in the immersion media following application of mineral trioxide aggregate (MTA) into root canals (pH values are expressed as mean ± SEM).

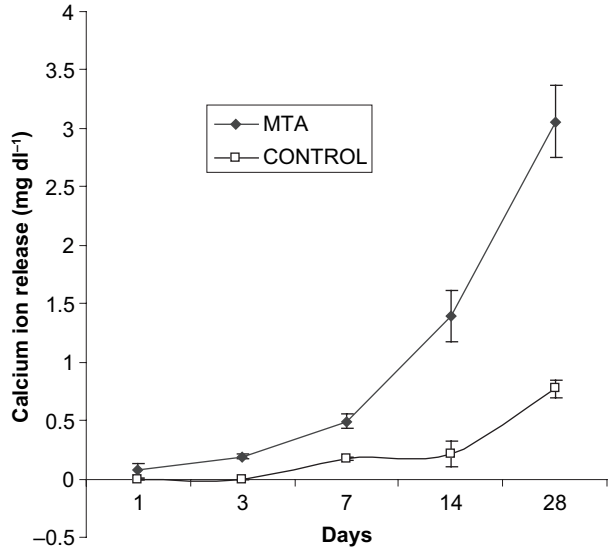

Fig. 3. Calcium ion release into the immersion media (mg dl^{-1}) following application of mineral trioxide aggregate (MTA) into root canals (values are expressed as mean ± SEM).

Ca^{+2} concentration (3–7 days) that remained almost stable between day 7 and day 14 and increased to 0.77 mg dl^{-1} at day 28 (Table 2). The Ca^{+2} concentration of the MTA group was significantly greater than that of the control values at all evaluation periods (Fig. 3, $P < 0.05$).

Discussion

Mineral trioxide aggregate has demonstrated favorable treatment outcome when used as a material for repairing root perforations at various locations (9, 16). As a root-end filling material, both freshly placed and set MTA promote dento-alveolar and osseous regeneration (17). This can be explained in part by the capability of MTA to activate cementoblasts to produce matrix formation by virtue of its better sealing ability, its high pH, or by releasing substances that activate cementoblasts (1, 3, 18–20). Moreover, MTA preferentially induces alkaline phosphatase expression and activity in both periodontal ligament and gingival fibroblasts (21). Torabinejad et al. (1) reported that calcium and phosphorus are the main ions in the composition of MTA. Although Ca^{+2} may seem to play a lesser role by activating Ca^{+2}-dependent adenosine triphosphatase in the repair potential of surrounding tissues, it is necessary in cell migration, differentiation, and mineralization (22, 23). Moreover, in order to stimulate mineralization, the material should also release calcium, which reacts with the tissue carbonic gas, forming calcium carbonate which favors mineralization (24). In light of these data, the present study was conducted to determine the possible release of calcium ions through dentinal tubules after intracanal MTA application.

In the present study, a significant, time-dependent increase in Ca^{+2} release was observed from day 3 to day 28. In light of the published data and the Ca^{+2} release values obtained herein, it is tempting to speculate that the time-dependent calcium ion release by MTA may favorably contribute to the repair process, when used behind a dentin barrier, such as in root resorptions. In the present study, the depth of the cavity was set to 1 mm, leaving a considerable amount of sound radicular dentin that could act as a barrier to the release of MTA. Nevertheless, no resorption cavity is standardized in the real situation. Further studies are required to investigate the effect of remaining dentin thickness on the release of Ca^{+2} from MTA.

One methodological concern is the localization of the simulated resorption defects. Because the cavity was prepared on the outer mid-root surface, the dentinal tubules were expected to be oriented at (approximately) 90° to the base of the defect. This value represents a mean angulation that may shift to a more oblique angle toward the apical direction. With the remaining dentin thickness kept constant, the distance of the tubular pathway for the release of ions would relatively increase as the angulations of the tubules become more oblique. Thus, further investigations must also incorporate comparisons at different levels of the root to draw strict conclusions. In the present study, MTA did not produce an alkaline shift in the immersion media. The pH values were confined to 7.4 (approximate value) during the entire test period. This finding can be explained by the permeability and buffering capacity of dentin, which may significantly affect the diffusion of hydroxyl ions (OH^-) through root dentin (23, 25). Therefore, when used as an indirect orthograde material, MTA should not be expected to contribute to healing by virtue of its high pH.

The results obtained within the experimental conditions of the present study are suggestive of a potential use of MTA in the case of inflammatory root resorption. However, further studies are required before beneficial

indirect effects of MTA can be advocated. These studies are underway.

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