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Calcium ion diffusion levels from MTA and apexcal in simulated external root resorption at middle third of the root

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Abstract – The purpose of this study was to investigate diffusion of calcium ions through exposed dentinal tubules following intracanal application of MTA and ApexCal. Thirty extracted anterior teeth were divided into three groups ($n = 10$): Group I: root canal prepared teeth with unfilled canals served as control; Group II: root canal space filled with MTA; Group III: root canal space filled with ApexCal. The teeth were decoronated, and root canal prepared to its working length and irrigated with EDTA and NaOCl. To simulate external root resorption, defects were created on the external surface of the root at the middle thirds. MTA/ApexCal was introduced into the canal and entrance sealed with IRM. The teeth were then coated with varnish except at the defect. Each tooth was immersed in a vial containing deionized water after which the release of Ca^{2+} from the defects into the deionized water was measured at 1, 7, 12, 14, and 28 days. Ca^{2+} concentrations of the immersion media were measured using a spectrometer. The post hoc-Bonferroni Alpha test (with mean difference significant at the 0.05 level) was done to statistically analyse the results using SPSS 11.5 software for Windows. The results showed greater calcium release by ApexCal than MTA with a significant increase with time. Within the experimental conditions of the present study APEX CAL may potentially be used in cases of root resorption.

Introduction

Resorption is a multifactorial process resulting in loss of tooth structure and most often an improper diagnosis of this condition has resulted in complete loss of the calcified tissues of the tooth. It has been a perplexing problem for all clinicians as its etiological factors are vague. Glossary of Contemporary Terminology for Endodontics (1) has defined resorption as a condition associated with either a physiologic or a pathologic dissolution of mineralized tissues such as dentin, cementum and/or bone resulting in a loss of these tissues by osteoclasts and osteoclast like cells. Gulabivala et al. (2) have reported that external root resorption (ERR) and Internal root resorption (IRR) can occur because of some of the common etiological factors like impacted teeth, luxation injuries, periapical inflammations due to necrotic pulp, periodontal diseases, bleaching endodontically treated tooth, radiation therapy, systemic diseases and tumours and cysts resulting in the discontinuity of organic outer cementoid and inner predentin layer which protects the root It is transient or progressive depending on the nature of the inflammatory stimulus.

If stimulated by bacteria, either in an infected pulp space or sulcus, the resorptive process will continue until the tooth is eventually lost. However, if the infective stimulus is removed, or the susceptible root surface is repaired, healing can take place. Calcium based materials have been widely used to induce healing or repair in root resorption defects.

Hence the purpose of this study was to investigate the diffusion of calcium ions (Ca^{2+}) through exposed dentinal tubules following intracanal application of Pro-Root™ MTA (mineral trioxide aggregate) and Apex Cal at middle thirds of the root.

Materials and methods

Thirty extracted maxillary anterior human teeth were collected, cleaned of debris and soft tissue remnants, inspected under an operational microscope for cracks or other defects 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 cemento-enamel junction using a slow-speed diamond disk. Then the root canals were instrumented by crown down technique with K-files till a master apical file size of 60 at the established working lengths. During cleaning and shaping, irrigation was done using a total of 5 ml 2.5% NaOCl at each file 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 canal instrumentation and cavity preparation, radiographs of the specimens (Fig. 1) were obtained with a paralleling technique (at 70 kVp and 0.4 s exposure). 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 teeth were then divided into three groups: Group I: teeth with defect at middle third with unfilled canals ($n = 10$) served as control group; Group II: teeth with defect at middle third filled with Pro-Root™ MTA (MTA) ($n = 10$); Group III: teeth with defect at the middle third filled with Apex Cal ($n = 10$).

The powder of MTA (Pro-Root™ MTA, Dentsply; Tulsa Dental, Tulsa, OK, USA) was mixed with deionised water supplied by the manufacturer 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 Pro-Root™ MTA (MTA) mixture was further condensed with hand pluggers so as to facilitate better adaptation of the material to root canal walls. Calcium and phosphorus are the main ions in the composition of MTA. MTA has two specific phases, comprising calcium oxide and calcium phosphate. Similarly ApexCal™ (Ivoclar Vivadent AG, Bendererstrasse 2, Principality of Liechtenstein) was also introduced into the canal space using the needle provided by the manufacturer. ApexCal contains calcium hydroxide and bismuth carbonate in a mixture of water, glycerine, polyethylene glycol and auxiliary materials. Finally, the coronal access was sealed with Intermediate Restorative Material (IRM, DENTSPLY Caulk, Milford, DE, USA). The entire root surfaces, except the external defects, were then coated with three coats of nail varnish. After self-drying of the varnish, samples was immersed in separate vials containing 20 ml deionized water, and stored at room temperature. The Ca^{2+}concentrations of the immersion media were measured at 1, 7, 12, 14 and 28 days using Perkin Elmer Optima 5300 DV ICP Optical Emission spectrometer (Fig. 2). $Ca^{2+}$was evaluated at a spectrum of 317.933 nm. The post hoc-Bonferroni Alpha test (with mean difference significant at the 0.05 level) was done to statistically analyse the results using SPSS 11.5 software for Windows.

Results

The mean thickness as determined by the paralleling radiographic technique employed was 1.49 ± 0.12 mm. There is an increase in the Ca^{2+}ion diffusion into the deionized water in all the three groups. Initially day 1 did not show a significant difference in the Ca^{2+}concentration between the groups ($P > 0.05$). But there was a significant increase in Ca^{2+}diffusion levels on days 7, 12, 14, 28 ($P < 0.05$). There was a significant increase in the Ca^{2+}ion diffusion in the ApexCal group as compared to the Pro-Root™ MTA (MTA) group ($P < 0.05$). The control group also showed a significant increase in the Ca^{2+}ion diffusion from day 1 to day 28 ($P < 0.05$). (Table 1, Fig. 3).

Discussion

Treatment of root resorption requires elimination of the inflammatory process and inhibition of the activity and formation of resorbing cells. Frank & Weine proposed treatment (3) for internal or external root resorption has been limited to the use of calcium hydroxide or other biomaterials to lessen the inflammatory response and to help promote deposition of a hard tissue barrier, while providing a biologic seal. Calcium hydroxide was introduced into dentistry in 1920 by Herrmann (20). It is a starkly alkaline substance with a pH value of 12.5. In an aqueous solution, Ca $(OH)_2$ dissociates into calcium and hydroxyl ions. Foreman and Barnes (4) reported that the bactericidal and osteogenic potential of calcium hydroxide is widely utilized in endodontics. Similarly, Holland et al. (5) described that calcium hydroxide creates an alkaline environment in the surrounding tissue where osteoclastic activity becomes impossible and hard tissue formation can take place. 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. The antimicrobial

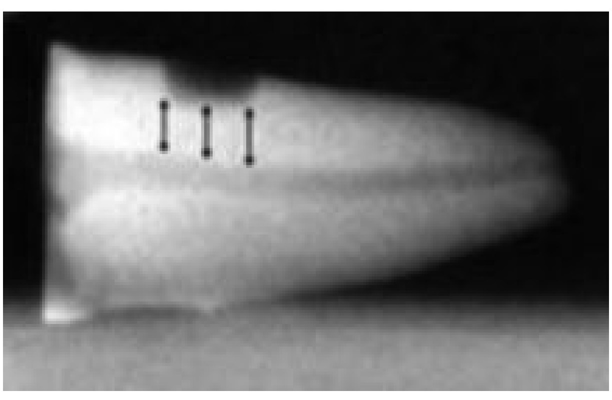

Fig. 1. Radiographs of the specimen showing remaining dentin thickness (1.49 ± 0.12 mm).

Fig. 2. Perkin-Elmer Optima 5300 DV ICP Optical Emission spectrometer.

Table 1. Release of calcium ions within time (mean values expressed as mg/l with standard deviation)

Days	Group I (mg/l), SD		Group II (mg/l), SD		Group III (mg/l), SD	
1	0.386	0.00057	0.471	0.00070	0.669	0.00066
7	2.702	0.00057	3.297	0.00057	4.684	0.00079
12	4.633	0.00067	5.652	0.00048	8.030	0.00447
14	4.904	0.00054	5.905	0.00125	9.929	0.00057
28	6.796	0.00066	12.78	0.01101	13.94	0.00422

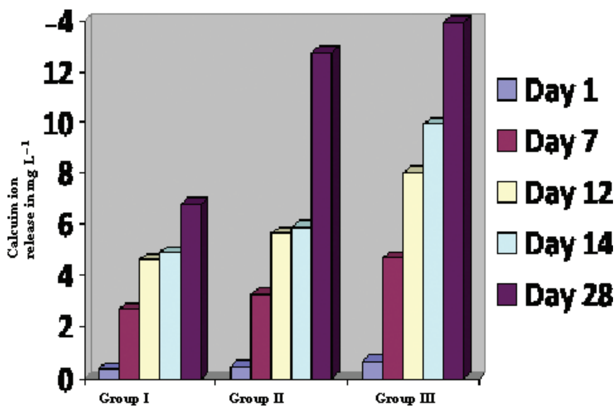

Fig. 3. Graph showing release of Ca^{2+}ions: X axis: groups. Y axis: Ca^{2+}ions in mg/l.

effect of calcium hydroxide is produced by the release of OH- ions and the subsequent increase in the pH of the surrounding dentin fluid. The pH value plays an important part in the growth, metabolism and cell division of microorganisms. A change in the concentration of OH- ions disturbs the pH gradient at the cell membrane and disrupts the energy supply of the bacteria, causing cells to die. Furthermore, Sbicego reported a high pH causes denaturation of cell membrane proteins and extracellular toxins (6).

Ruddle (7), Torabinejad et al. (8), Torabinejad et al. (9), Vizgirda et al. (10) have reported that Mineral trioxide aggregate (MTA) has been a biomaterial of considerable clinical and laboratory research. Besides its potential use as a root-end 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). Torabinejad et al. (8) have shown that calcium and phosphorus are the main ions in the composition of MTA. MTA has two specific phases, comprising calcium oxide and calcium phosphate. As per Holland et al. (11), Seux et al. (12) and Torabinejad et al. (8) the calcium oxide reacts with tissue fluids to form calcium hydroxide. Fridland & Rosado (13) reported that calcium (in its 'hydroxide' form) is the main chemical compound released by MTA in water. Estrela et al. (14) have shown that 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, release of $Ca^{2+}$from MTA could yield a desirable healing effect.

ApexCal contains calcium hydroxide and bismuth carbonate in a mixture of water, glycerine, polyethylene glycol and auxiliary materials. ApexCal has a pH of around 12.4. Compared to other commercially available preparations, ApexCal showed a very homogenous and constant consistency over time. Sbicego (6) reported that ApexCal is easy to pick up with lentulo spirals, yet easy to spin into the canals because of its excellent stability.

The present study was conducted to determine the possible release of calcium ions through dentinal tubules after intracanal application of Calcium ion releasing materials. Calt et al. (15) has reported that 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. Javalet et al. (16) have suggested that in order to stimulate mineralization, the material should also release calcium, which reacts with the tissue carbonic gas, forming calcium carbonate which favours mineralization. One study by Özdemir et al. (17) shows that 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 through root dentin. Özdemir et al. (17) have also reported that when used as an indirect orthograde material, MTA should not be expected to contribute to healing by virtue of its high pH.

This study indicates that non-setting Calcium releasing materials have an effective release of Ca^{2+}ions compared to MTA. Thus in this study we have seen that diffusion of Ca^{2+}ions from ApexCal were greater than that of Pro-Root™ MTA (MTA). Being a paste it will be in good contact with the dentin surface, and the calcium hydroxide can better exert its antibacterial effects. Beer (18) has suggested that using glycerine paste, Calcium diffusion was seen to be 50% greater in comparison to the aqueous mixture. On the other hand MTA is a powder which once set release Ca^{2+}at a constant regulated level. This could be the reason why ApexCal gave a greater Ca^{2+}diffusion rate than MTA. Beer (18) has reported that calcium hydroxide mixtures with water were less homogenous and thereby resulted in lesser release of Ca^{2+}ions.

The release of Ca^{2+}ions in the control group may be from the leaching out of Ca^{2+}ions from the tooth which is a dynamic structure. Vogel et al. (19) showed that enamel and dentin have a positive membrane while cementum is negative. This chemical property hinders diffusion of hydroxyl ions through the dentin while Ca^{2+}diffusion is prevented by the cemental layer. When there is a defect on the root surface, a disruption of the negative membrane potential occurs there by allowing Ca^{2+}diffusion. In light of the Ca^{2+}release values obtained herein, it is tempting to speculate that the time-dependent calcium ion release by Pro-Root™ MTA (MTA) and ApexCal may favourably 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 Ca^{2+} from Pro-Root™ MTA (MTA) and ApexCal. In the real situation no resorption cavity can be standardized. 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 Ca^{2+}. The problem with *in vitro* measurements, however, is that few definitive conclusions regarding the actual clinical situation can be drawn. The mean remaining dentin thickness in this study was 1.49 ± 0.12 mm. 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 these materials. 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. Similarly, further clinical studies are required before beneficial effects of ApexCal can be advocated.

Conclusion

The results obtained within the experimental conditions of the present study are suggestive of a potential use of APEX CAL and Pro-Root™ MTA (MTA) in the case of inflammatory root resorption. Ca^{2+} ion diffusion from APEX CAL was significantly greater than Pro-Root™ MTA (MTA)and therefore can be used as an intracanal medicament for treatment of external inflammatory root resorption.

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