

Original Article

Application of 17% EDTA Enhances Diffusion of ^{45}Ca -labeled OH^- and Ca^{2+} in Primary Tooth Root Canal

Marcos Ximenes¹⁾, Carolina Mayumi Cavalcanti Taguchi¹⁾,
Thaís Cezária Triches²⁾, Neimar Sartori³⁾, Luis Alberto Pereira Dias⁴⁾,
Elaine Bortoleti de Araujo⁵⁾ and Mariane Cardoso¹⁾

¹⁾ School of Dentistry, Federal University of Santa Catarina,
Rua: Manoel Severino de Oliveira,
185-106, CEP 88062120, Florianópolis, Santa Catarina, Brazil

²⁾ Private Practice

³⁾ Division of Restorative Sciences, Herman Ostrow School of Dentistry,
University of Southern California,
925 West 34th Street, Los Angeles, CA 90089-0641, USA

⁴⁾ Department of Radioisotopes Production, Radiopharmacy Directory,
Research Institute and Nuclear Energy, Avenida Lineu Prestes,
2242, Cidade Universitária, CEP 05508-000, São Paulo, Brazil

⁵⁾ Department of Quality Assurance, Radiopharmacy Directory,
Research Institute and Nuclear Energy, Avenida Lineu Prestes,
2242, Cidade Universitária, CEP 05508-000, São Paulo, Brazil

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Abstract

Proper cleaning of the root canal is key to the success of endodontic treatment as it allows more effective diffusion of medication throughout the dentinal tubules. The aim of this *in vitro* study was to investigate the efficacy of 17% ethylenediaminetetraacetic acid (EDTA) in enhancing diffusion of hydroxyl (OH^-) and calcium ions (Ca^{2+}) throughout the root canal in primary teeth. The canals of 25 primary tooth roots were cleaned with endodontic files and 1% sodium hypochlorite. Three groups (G) were then established: GI, in which final irrigation was performed with 1% sodium hypochlorite; GII, in which 17% EDTA was used; and GIII, in which no irrigation was performed. The roots canals in GI and GII were filled with a calcium hydroxide-based paste labeled with the radioisotope calcium-45. Diffusion of OH^- was detected with pH strips and Ca^{2+} analyzed by measuring radioactivity in counts per min. Group II differed statistically from the other groups in diffusion of OH^- at 24 hr ($p < 0.05$), but no significant difference among groups was found at the day 7 evaluation; GII also differed statistically from the other groups in diffusion of Ca^{2+} at 24 hr ($p < 0.05$). These results suggest that application of 17% EDTA in primary tooth enhances diffusion of OH^- and Ca^{2+} .

Key words: Edetic acid — Radioisotopes — Calcium hydroxide — Tooth — Deciduous

Introduction

The aim of endodontic treatment in primary teeth is elimination of infection and conservation until physiological exfoliation and replacement with a permanent successor can take place. Thus, health, function, and esthetics are maintained. Cleaning and disinfection of the root canal are key to the success of a pulpectomy. The proper combination of dental instruments, irrigation with effective solutions, temporary dressing, and correct filling of the root canal increase the odds of success¹⁴⁾.

The chelating agent ethylenediaminetetraacetic acid (EDTA) can be used to remove the smear layer formed during the chemical-mechanical preparation of the canal^{15,19)}. The presence of this smear layer can compromise the outcome of treatment, as it serves as a shelter for microorganisms in the dentinal tubules and blocks the action of medication, exerting a negative influence on the prognosis²¹⁾. The removal of the smear layer allows greater permeability of the dentin and greater diffusion of intra-canal medication²⁾.

Calcium hydroxide is also used as an intra-canal medication in the endodontic treatment of infected primary teeth due to its antiseptic action and ability to stimulate conditions favorable to tissue repair²²⁾. In the presence of water, calcium hydroxide breaks down into hydroxyl ions (OH^-) and calcium ions (Ca^{2+}), which are responsible for its therapeutic action. Diffusion of OH^- raises the pH^{7,13)}, destroying bacteria and inactivating their enzymes, while that of Ca^{2+} reduces osteoclastic activity and activates alkaline phosphatase, which is involved in tissue re-mineralization⁴⁾.

A 17% EDTA solution is a common choice in treating root canals in primary teeth^{1,11)}. However, few studies have investigated its efficacy and applicability in conjunction with calcium hydroxide in the endodontic treatment of these teeth.

The use of radioisotopes and radioactivity analysis is a sensitive technique for determining diffusion of drugs throughout dentinal

tubules¹⁶⁾. Calcium-45 (^{45}Ca) is the most commonly used, because it is a low-energy beta emitter and does not readily penetrate enamel. Radioactive isotopes provide finer detail in diffusion studies as the small isotope molecules measure only 40 nm and can penetrate spaces of only 1–2 μm ⁹⁾.

The aim of this *in vitro* study was to investigate the efficacy of 17% EDTA in diffusing ^{45}Ca -labeled OH^- and Ca^{2+} throughout the root dentin of primary teeth. The results were compared with those obtained by using sodium hypochlorite 1% for the final irrigation. The hypothesis was that irrigation with 17% EDTA would result in greater diffusion throughout the tooth root.

Materials and Methods

1. Ethical considerations

The protocol of this study was approved by the Human Research Ethics Committee of this institute (Approval number: 750).

2. Sample

Twenty-five primary tooth roots from maxillary and mandibular incisors and molars were obtained through transverse sectioning with a double-sided diamond-cutting disk (number 7016, KG Sorensen®, São Paulo, Brazil) 2.0 mm short of the cemento-enamel junction. At least two thirds of each root was left intact. There was no resorption in the apical, medial, or cervical third; no calcification; and no accentuated curvature.

3. Preparation of roots

The length of each root was standardized to 7 mm and the apices sealed with Super Bonder Gel (Loctite®, São Paulo, Brazil). Each canal was individually cleaned, first with a series of dental files (Dentsply-Maillefer®, São Paulo, Brazil), beginning with the file that best fit the canal, and then with two subsequent files. Cleaning was performed throughout the 7 mm working length, making 15 circumferential movements with each file. A new set of files was used for each set of

roots. The canal was irrigated with 3 ml of 1% sodium hypochlorite solution (Miyako[®], São Paulo, Brazil) and simultaneously aspirated.

The specimens were then randomly divided into 3 experimental groups (G): GI (n = 10), in which final irrigation was performed with 1 ml of 1% sodium hypochlorite for 3 min; GII (n = 10), in which final irrigation was performed with 1 ml of 17% EDTA (Biodinâmica, Ibiporã, Paraná, Brazil) for 3 min; and GIII (n = 5), in which no final irrigation was performed. The canals were dried with absorbent paper points (Dentsply-Maillefer[®]) and bathed in ultrasound (Ultrasonic 1440[®]-Odontobrás, São Paulo, Brazil) for 15 min to remove any remaining sodium hypochlorite or 17% EDTA.

4. Preparation of medication

The calcium hydroxide-based solution was prepared in a chamber, mixing 200 μ l calcium hydroxide paste (SS White[®], Rio de Janeiro, Brazil) with 500 μ l ⁴⁵Ca (Radiofarmácia-IPEN, São Paulo, Brazil). The mixture was blended by using a glass stirrer until a homogeneous fluid solution was obtained. The solution was then left to rest for 5 min. A 1-ml hypodermic syringe was used to deliver the final solution to the canals. Only the specimens in GI and GII received 10 μ l calcium hydroxide/⁴⁵Ca solution; GIII was maintained as a negative control. Wax (Dentsply-Maillefer[®]) was used to seal the entrance of the root canal. Restoration was performed over the seal with a light-curing resin composite (Opallis[®], FGM, Santa Catarina, Brazil).

The crown region of each specimen was attached to a metal rod by means of a light-curing resin composite. The rod was made of orthodontic wire 0.6 mm in thickness and 3 cm in length. To simulate the level of pH in periapical lesions, which is reported to be within the range of 6.00 to 7.03¹²⁾, boric acid was added to saline solution to achieve a pH of approximately 6.00. Each specimen was then placed in a 1.8-ml acrylic recipient containing 1 ml saline solution (pH = 6.00), which was replaced after each evaluation. All specimens remained immersed in this solution, and a constant temperature of 37°C

and 100% relative humidity were maintained throughout the experiment.

5. Determination of pH

The pH of the medium (saline solution) was determined using pH strips (Merk[®], Darmstadt, Germany) inserted individually into each recipient. Readings were taken at regular intervals in all groups (initial, 24 hr, 3 days, and 7 days). The pH values were recorded based on the coloration of the strips and the standard measure.

6. Determination of radioactivity

After each pH reading, diffusion was investigated by determining the level of radioactivity in the specimen-containing saline solution. Each specimen was removed from the recipient and the solution poured into a test tube placed in a Gamma Counter (D5002, Cobra II, Autogamma, Canberra Packard, Meriden, Connecticut, USA) to measure radioactivity in counts per minute (CPM) for 1 min.

7. Statistical analysis

The SPSS 19 program (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analysis. The Friedman and Wilcoxon tests were used to determine differences in pH at each sample evaluation. A repeated-measures analysis of variance (ANOVA) and the Bonferroni test were used to determine differences in diffusion of Ca²⁺. The Kruskal-Wallis and Mann-Whitney tests were used to determine differences in pH between groups. A one-factor ANOVA and the Dunnett and Tukey HSD tests were used to determine differences in diffusion of Ca²⁺ between groups.

Results

As shown in Fig. 1, release of a significantly greater amount of OH⁻ was observed at the 24-hr evaluation in GII than in the other groups (p = 0.008). Although statistically significant differences were found among the three groups at the day 3 evaluation (p = 0.002), none was found at the day 7 evaluation

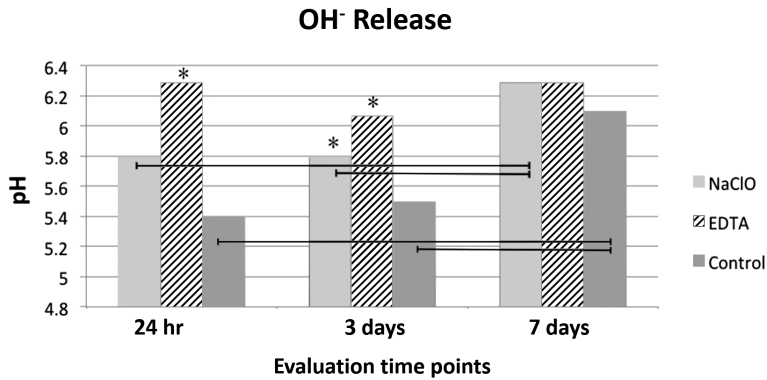


Fig. 1 Schematic of OH⁻ release in each group over 3 time points assessed. Values linked by bar are statistically different ($p < 0.05$). Values with asterisk represent statistical difference with control group ($p < 0.05$).

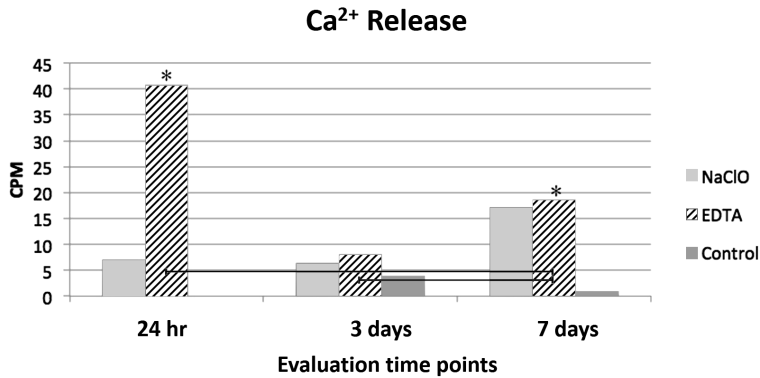


Fig. 2 Schematic of Ca²⁺ release in each group over 3 time points assessed. Values linked by bar are statistically different ($p < 0.05$). Values with asterisk represent statistical difference with control group ($p < 0.05$).

($p = 0.303$). The intra-group analysis revealed statistically significant differences in GI and GIII, with a greater amount of OH⁻ released at the day 7 evaluation; no statistically significant differences were found among the three time points in GII, however ($p = 0.261$).

Figure 2 shows the results for release of Ca²⁺ in all groups. A significantly greater amount of Ca²⁺ was released in GII than in the other groups at the 24-hr evaluation ($p < 0.001$). No statistically significant difference was found among the three groups at the day 3 evaluation ($p = 0.524$). A significant difference was observed between GII and GIII

at the day 7 evaluation ($p = 0.042$), but both groups were statistically similar to GI. The intra-group analysis revealed no statistically significant difference in GI ($p = 0.107$) or GIII ($p = 0.152$) among the three time points; release of Ca²⁺ was significantly greater at the 24-hr evaluation in GII, however ($p < 0.001$).

Discussion

Radioisotopes are unstable atoms that disintegrate, giving off radiation. In this study, a radioisotope method was selected rather than

a chemical one as emission of radiation makes them easy to detect¹⁾, even when present in only very low quantities. The isotope selected, the low energy beta emitter ⁴⁵Ca, showed selective and deep diffusion into the dentinal tubules.

The cervical and apical portions of the specimens were sealed, so the only means of passage were the dentinal tubules and cementum, proving that OH⁻ and Ca²⁺ were released through the root, which is similar to findings reported in previous studies with primary^{3,14)} and permanent teeth^{20,24)}.

To assess diffusion of OH⁻ and Ca²⁺, the roots were immersed in saline solution (pH=6.00). The length of immersion was established in reference to clinical reports on periradicular lesions^{17,24)}. Unlike in other studies^{14,21)}, however, here the saline solution into which the root was immersed was replaced after each evaluation to allow maximal diffusion of ions without saturation of the medium⁸⁾.

The minimal amount of OH⁻ required to make the medium unviable for bacterial proliferation (neutralization of the acidic medium) remains to be established. Therefore, in alkalinizing the medium, it is desirable to use a material that releases the largest possible number of these ions^{3,23)}. A more acidic external medium results in greater diffusion of OH⁻ and a subsequent tendency toward neutralization, which may occur in inflammatory processes related to the roots of primary teeth.

The antimicrobial action of calcium hydroxide is related to the alkaline pH. Change in dentinal pH due to the presence of OH⁻ is slow and depends on several factors that can alter the rate of ionic dissociation and diffusion, such as the level of hydrosolubility of the vehicle employed, difference in viscosity, acid-base characteristics, dentinal permeability, and level of existing calcification^{6,10)}. In the analysis of OH⁻ diffusion, GII showed the highest pH values at all evaluation times, with statistically significant differences in relation to the other groups at 24 hr and 3 days. This finding demonstrates that removing the

smear layer with 17% EDTA was effective in enhancing the permeability of the dentinal tubules in the first few days following application of intra-canal medication. Moreover, it suggests that this approach may be more effective than application of calcium hydroxide-based medication. Similar findings have been reported by other authors^{5,18)}, who noted that removing the smear layer with irrigating solutions such as 17% EDTA or 5.25% NaOCl facilitated diffusion of OH⁻ throughout dentinal tubules. To the best of our knowledge, no studies to date have shown that use of 17% EDTA in primary teeth is harmful to the permanent teeth.

At the end of the 7-day analysis period, no statistically significant difference in pH was observed among the three groups. Çalt *et al.*³⁾ found a reduction in pH throughout the analysis period in all groups tested. According to the authors, the permeability and buffering capacity of the dentin are key factors that directly affect the diffusion of OH⁻ through the root dentin. Tanomaru-Filho *et al.*²⁰⁾ found greater amounts of OH⁻ in the first 12 hr of their study, with all groups exhibiting similar pH values at the end of a 7-day analysis. This suggests that 7 days is the approximate amount of time necessary for a significant volume of material to pass through the dentinal tubules without prior removal of the smear layer. This demonstrates the efficiency of 17% EDTA in accelerating diffusion of calcium hydroxide-based medication.

Release of Ca²⁺ was observed in all groups at some point. A significantly greater amount of Ca²⁺ was released in GII than in the other groups at 24 hr, demonstrating the efficacy of 17% EDTA in removing the smear layer. This is in agreement with findings reported by other authors, who, despite observing peak diffusion at 7 days, found a high degree of diffusion on the first day of analysis^{3,18)}. However, in disagreement with these results, Nunes and Rocha¹⁴⁾, using an atomic absorption spectrophotometer unit, found greater diffusion of Ca²⁺ after 7 days, with maximal values reached at day 30. This disparity may be related to differences in the methodologies

employed in these studies. In the present study, the CaOH solution was mixed with $500\ \mu\text{l}$ ^{45}Ca , resulting in lower viscosity and, therefore, better flow of the solution. This can be seen in the higher rates of Ca^{2+} release. The time needed for calcium hydroxide to optimally disinfect the root canal is still unknown and might be related to the type of microorganism involved, location of the microorganism in the root canal system, presence or absence of smear layer, and presence or absence of root canal exudation^{14,15}. Despite not achieving statistical significance in the intra-group analysis, peak release of Ca^{2+} occurred on day 3 in GIII. This may be attributed to the fact that the tooth itself is capable of releasing calcium from its own structure, as reported in previous studies^{3,23}.

This study had some limitations. Although an attempt was made to approximate clinical conditions by using extracted human teeth selected in a standardized manner, the results are research-based and should therefore be extrapolated with care. As described above, the consistency of intra-canal medication used clinically may vary. Moreover, as with any medication or substance used inside the primary tooth canal, biocompatibility with the germ of the permanent tooth needs to be clarified.

Conclusion

Using 17% EDTA to clean the root canal in primary teeth resulted in an increase in the diffusion of OH^- and Ca^{2+} .

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References

- 1) Bello-Silva MS, Lage-Marques JL, Marotti J, de Paula Eduardo C, Apel C, Gutknecht N (2010) Calcitonin, sodium alendronate and high intensity laser in the treatment of traumatized teeth: a preliminary study. *Lasers Med Sci* 25: 331–337.
- 2) Bystrom A, Sundqvist G (1985) The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J* 18:35–40.
- 3) Çalt S, Serper A, Özçelik B, Dalat MD (1999) pH changes and calcium ion diffusion from calcium hydroxide dressing materials through root dentin. *J Endod* 25:329–331.
- 4) Canoglu H, Tekcicek MU, Cehreli ZC (2006) Comparison of conventional, rotary, and ultrasonic preparation, different final irrigation regimens, and 2 sealers in primary molar root canal therapy. *Pediatr Dent* 28:518–523.
- 5) Chamberlain TM, Kirkpatrick TC, Rutledge RE (2009) pH changes in external root surface cavities after calcium hydroxide is placed at 1, 3 and 5 mm short of the radiographic apex. *Dent Traumatol* 25:470–474.
- 6) Estrela C, Pimenta FC, Ito IY, Bammann LL (1998) *In vitro* determination of direct antimicrobial effect of calcium hydroxide. *J Endod* 24:15–17.
- 7) Felipe MCS, Felipe WT, Espezim CS, de Freitas SFT (2006) Effectiveness of NaOCl alone or in combination with EDTA on the diffusion of hydroxyl ions released by calcium hydroxide paste. *J Appl Oral Sci* 14:1–5.
- 8) Foster KH, Kulild JC, Weller RN (1993) Effect of smear layer removal on the diffusion of calcium hydroxide through radicular dentin. *J Endod* 19:136–140.
- 9) Going RE, Massler M, Dute HL (1960) Marginal penetration of dental restorations by different radioactive isotopes. *J Dent Res* 39:273–284.
- 10) Gomes BP, Ferraz CC, Vianna ME, Rosalen PL, Zaia AA, Teixeira FB, Souza-Filho FJ (2002) *In vitro* antimicrobial activity of calcium hydroxide pastes and their vehicles against selected microorganisms. *Braz Dent J* 13:155–161.
- 11) Hariharan VS, Nandlal B, Srilatha KT (2010) Efficacy of various root canal irrigants on removal of smear layer in the primary root canals after hand instrumentation: a scanning electron microscopy study. *J Indian Soc Pedod Prev Dent* 28:271–277.
- 12) Nekoofar MH, Namazikhah MS, Sheykhrezae MS, Mohammadi MM, Kazemi A, Aseeley Z, Dummer PM (2009) pH of pus collected from

- periapical abscesses. *Int Endod J* 42:534–538.
- 13) Nerwich A, Figdor D, Messer HH (1993) pH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide. *J Endod* 19:302–306.
 - 14) Nunes ACGP, Rocha MJ de C (2005) Hydroxyl and calcium ions diffusion from endodontic materials through roots of primary teeth — *in vitro* study. *J Appl Oral Sci* 13:187–192.
 - 15) Pascon FM, Kantovitz KR, Sinhoretí MA, Puppim-Rontani RM (2007) Is the presence of the smear layer a limiting factor for root dentin permeability in primary teeth? *J Dent Child (Chic)* 74:182–188.
 - 16) Puckett AD, Fitchie JG, Bennett B, Hembree JH (1995) Microleakage and thermal properties of hybrid ionomer restoratives. *Quintessence Int* 26:577–581.
 - 17) Ruvière DB, Leonardo MR, da Silva LAB, Ito IY, Nelson-Filho P (2007) Assessment of the microbiota in root canals of human primary teeth by checkerboard DNA-DNA hybridization. *J Dent Child (Chic)* 74:118–123.
 - 18) Saif S, Carey CM, Tordik PA, McClanahan SB (2008) Effect of irrigants and cementum injury on diffusion of hydroxyl ions through the dentinal tubules. *J Endod* 34:50–52.
 - 19) Sum CP, Neo J, Kishen A (2005) What we leave behind in root canals after endodontic treatment: some issues and concerns. *Aust Endod J* 31:94–100.
 - 20) Tanomaru-Filho M, Chaves Faleiros FB, Saçaki JN, Hungaro Duarte MA, Guerreiro-Tanomaru JM (2009) Evaluation of pH and calcium ion release of root-end filling materials containing calcium hydroxide or mineral trioxide aggregate. *J Endod* 35:1418–1421.
 - 21) Teixeira CS, Felipe MC, Felipe WT (2005) The effect of application time of EDTA and NaOCl on intracanal smear layer removal: an SEM analysis. *Int Endod J* 38:285–290.
 - 22) Tronstad L, Andreasen JO, Hasselgren G, Kristerson L, Riis I (1981) pH changes in dental tissues after root canal filling with calcium hydroxide. *J Endod* 7:17–21.
 - 23) Ximenes M, Cardoso M (2012) Assessment of diffusion of hydroxyl and calcium ions of root canal filling materials in primary teeth. *Pediatr Dent* 34:122–126.
 - 24) Zampronio CF, Sivieri-Araújo G, Bonetti-Filho I, Berbert FL (2008) pH changes after manual or ultrasonic instrumentation and smear layer removal with EDTA or ultrasonic. *Dent Traumatol* 24:542–545.

Correspondence:

Dr. Marcos Ximenes
 School of Dentistry,
 Federal University of Santa Catarina
 Rua: Manoel Severino de Oliveira,
 185-106, CEP 88062120, Florianópolis,
 Santa Catarina, Brazil
 E-mail: marcos.ximenes@ufsc.br