Ethylenediaminetetraacetic Acid and Citric Acid Solutions for Smear Layer Removal in Primary Tooth Root Canals

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ABSTRACT

Purpose: The purpose of this in vitro study was to evaluate, via scanning electron microscopy, the micromorphology of the dentine walls of primary anterior teeth with focus on the presence of the smear layer after endodontic debridement and final irrigation with different systems.

Methods: Thirty primary maxillary anterior teeth were assigned to 3 groups according to the final irrigant solution. Group 1 received 1% sodium hypochlorite; Group 2 received 17% EDTA followed by 1% sodium hypochlorite; and Group 3 received 6% citric acid followed by 1% sodium hypochlorite. The canals were debrided using 1% sodium hypochlorite to a size 30 file, and the final irrigation was performed with one of the 3 irrigants as assigned. The teeth were prepared for examination of the canal walls by electron microscopy for the presence or absence of the smear layer.

Results: Group 1 had significantly higher scores for remaining smear layer than Groups 2 and 3 ($P<.01$), while Groups 2 and 3 presented similar smear layer score values.

Conclusions: Sodium hypochlorite promoted the formation of a smear layer during shaping, and the use of EDTA and citric acid facilitated smear layer removal.

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Endodontic treatment of primary teeth with necrotic pulps, or those irreversibly affected by an inflammatory process is of fundamental importance for avoiding consequences arising from infection. Among the consequences are early primary tooth loss and its repercussions, including damage to the permanent tooth during formation, due to dissemination of the infection to the periradicular tissues. This therapy must involve disinfecting the root canal system and filling with reabsorbable pastes.[1,2]

Root canal preparation is a fundamental step in endodontic treatment. However, primary teeth have zones inaccessible to debridement, such as accessory canals, ramifications, and dentinal tubules. Therefore, it is imperative to use auxiliary solutions that promote disinfection of these areas, mainly because infected primary teeth can harbor micro-organisms inside the dentinal tubules,[3] in the same way permanent teeth do.[4]

A way to open the dentinal tubules and allow medications to act inside them is to remove the smear layer formed during root canal debridement and irrigate the canal with solutions specifically for this purpose, such as ethylenediaminetetraacetic acid (**EDTA**) or citric acid. In the literature, one finds many studies evaluating the effectiveness of these substances when used during or at the end of biomechanical preparation of permanent teeth.[5-18] In primary tooth root canals, it has been reported that irrigating the canals with EDTA allows zinc oxide and eugenol-based filling paste to enter into the dentinal tubules and that citric acid is very effective for removing the smear layer.[19]

Thus, the purpose of this study was to evaluate, via scanning electronic microscopy, the micromorphology of the dentin walls of the root canals in primary teeth submitted

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to endodontic debridement in vitro, and with different auxiliary irrigant substances, focusing on the presence or absence of the smear layer.

METHODS

This study was approved by the Ethics Committee of the Federal University of Rio Grande do Sul School of Dentistry, Porto Alegre, Rio Grande do Sul, Brazil, under protocol no. 66/06. The sample consisted of 30 primary maxillary anterior teeth (central and lateral incisors and canines) which, on visual inspection, presented a minimum of two thirds of intact root. The tooth crowns were discarded and only the roots were manipulated.

The teeth were divided into 3 experimental groups of 10 each according to the final irrigant solution:

1. Group 1—irrigation with 3.6 ml of 1% sodium hypochlorite (Laboratório Inodon, Rio Grande do Sul) for 1 minute.
2. Group 2—irrigation with 1.8 ml of 17% EDTA (disodium EDTA p.a 17 g; 5 N sodium hydroxide solution 9.25 ml; distilled water qsp 100 ml manipulated by "Farmácia Calêndula," Rio Grande do Sul) for 1 minute; the solution remained inside the canal for 2 minutes, followed by irrigation with 1.8 ml of 1% sodium hypochlorite for 30 seconds.
3. Group 3—irrigation with 1.8 ml of 6% citric acid (citric acid in an aqueous solution manipulated by "Farmácia Calêndula") for 1 minute. The solution remained inside the canal for 2 minutes, followed by irrigation with 1.8 ml of 1% sodium hypochlorite for 30 seconds.

Irrigant substances were stored in empty plastic dental anesthetic tubes, and irrigation was performed with a Carpule syringe (Dufex) and disposable anesthetic needles (short 30 G, Ibrás-CBO, Campinas-Sao Paulo, Brazil). For aspiration, cannulas (40-20 gauge) (Ibrás-CBO) were coupled to the suction device and placed at the opening into the canal, and aspiration and irrigation were performed simultaneously.

BIOMECHANICAL ROOT CANAL PREPARATION

The working length of the canal was determined by introducing a small caliber file inside the root canal until it was seen in the apical foramen. Of the total measurement of the tooth established with this file, 1 mm was subtracted to give a working length 1 mm short of the apex.

Root canal debridement was performed with file numbers 15, 20, 25, and 30 in the entire working length of the canal. Between every instrument, irrigation was performed with 1.8 ml of 1% sodium hypochlorite and concomitant aspiration. The instrument was only exchanged for another of larger caliber when the previous one moved freely inside the canal.

Final irrigation was different in each experimental group. Group 1 used sodium hypochlorite; Group 2 used EDTA followed by sodium hypochlorite; and Group 3 employed citric acid followed by sodium hypochlorite. Drying was performed with absorbent paper cones until the canal was completely dry.

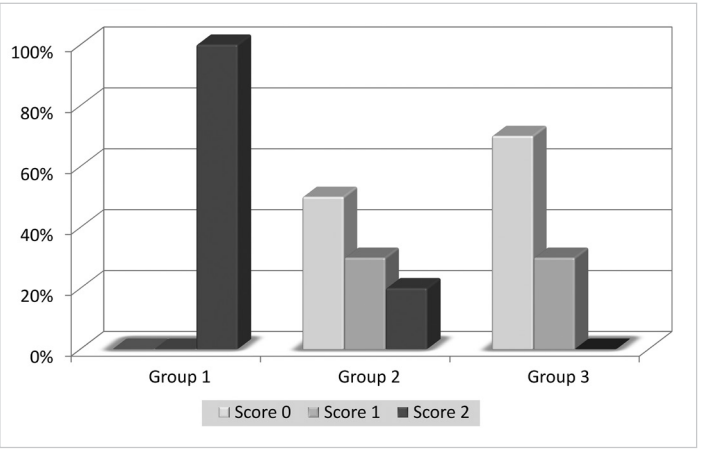

Figure 1. Frequencies relative to scores per treatment group (n=10) (P<.01).

After the experimental stage, the samples of each group were prepared for analysis by scanning electronic microcopy. The roots were split in the long axis to expose the internal surface, and only half of each randomly chosen root was evaluated.

Areas representative of the micromorphology of each tooth at 1,000x magnification were selected and printed on special paper, and the images were randomly numbered. Three professional endodontic specialists, informed about the parameters used for evaluation by means of an explanatory text, performed the analyses. They evaluated the presence or absence of the smear layer in accordance with the following criteria[20]:

Score 0=absence of smear layer (majority of dentinal tubules open);
Score 1=moderate presence of smear layer and outline of tubules partially visible (majority of tubules partially obliterated); and
Score 2=presence of abundant smear layer (majority of dentinal tubules completely obliterated).

For each image, which corresponded to one tooth, one of the aforementioned scores were attributed by each examiner and recorded on a chart. The evaluators did not know to which experimental group each image belonged.

Before the results were analyzed, agreement among the examiners was evaluated by the Friedman test (5%), and no statistically significant difference was observed ($P<.17$). The evaluators agreed with approximately 87% of the scores attributed.

For each image, the score most attributed among the 3 examiners was counted. The groups were then compared via the Kruskall Wallis test (5%). All the analyses were performed with SPSS 13 software (SPSS Inc, Chicago, Ill.)

RESULTS

The graph (Figure 1) contains the frequency distributions of the scores in each experimental group, considering the score most attributed to each image.

Multiple comparisons indicated that Group 1 differed statistically from the others, with higher score values (mean rank=24.50). Groups 2 and 3 presented similar score values (mean rank=12.4 and 9.6, respectively). In the groups in which EDTA or citric acid (decalcifying substances) were used, there was removal of the smear layer formed during debridement, while in Group 1 an abundant smear layer remained.

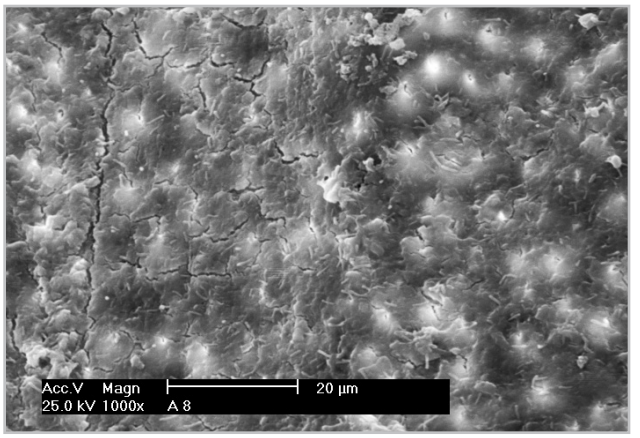

Figure 2. Photomicrograph of Group 1 revealing an abundant smear layer pattern (score 2).

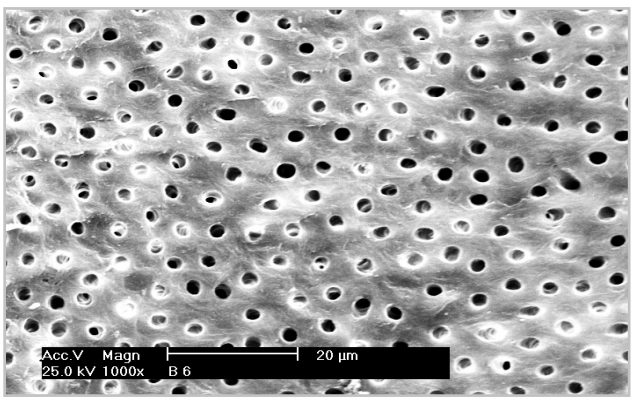

Figure 3. Photomicrograph of Group 2 showing open dentinal tubules (score 0).

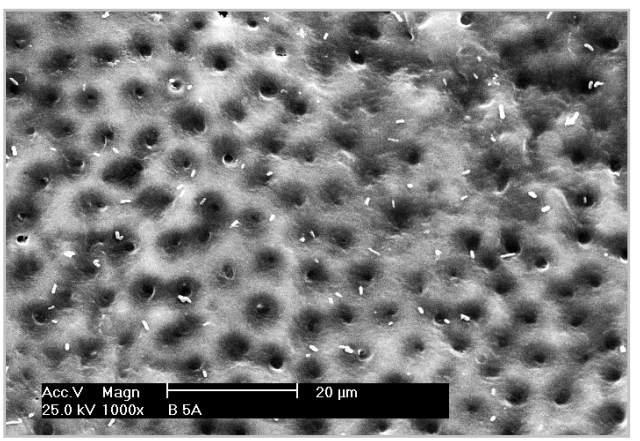

Figure 4. Photomicrograph of Group 2 indicating partially obstructed dentinal tubules (score 1).

In Group 1, in which 1% sodium hypochlorite as a single irrigant solution was used, the samples presented an abundant smear layer pattern, with completely obliterated dentinal tubules (Figure 2). In Group 2, the final irrigation system in which 17% EDTA + 1% sodium hypochlorite was used, most samples presented open dentinal tubules (Figure 3) and, secondarily, partially obstructed dentinal tubules (Figure 4). Few samples presented obliterated dentinal tubules. In Group 3, the final irrigation system in which 6% citric acid + 1% sodium hypochlorite was used, most samples presented an absence of a smear layer pattern (Figure 5), followed by some samples with partially obstructed dentinal tubules. No samples presented obliterated dentinal tubules.

DISCUSSION

The success of endodontic treatment in primary teeth with necrotic pulp lies in the biomechanical preparation, making it feasible to obtain a clean and disinfected canal in good conditions for filling.

Some studies have shown that there are microorganisms inside the dentinal tubules of root canals in primary and permanent teeth with infected or necrotic pulps.[3,21] As this is an area in which debridement is deficient, it would be important to make it feasible for the medications used during pulp therapy to act (biomechanical preparation, delayed dressings and filling materials) inside the dentinal tubules.[5,11]

The results obtained in this research agree with studies developed in permanent teeth, which demonstrated the presence of an abundant smear layer when only sodium hypochlorite was used during biomechanical preparation and as a final irrigant solution.[7,11,14,22-27] In primary teeth, a relative smear layer removal was observed after final irrigation with 5% sodium hypochlorite for 30 seconds. Although this was a high concentration, these data differ completely from the literature on permanent teeth. This did not occur when irrigation lasted only 15 seconds or when an abundant smear layer remained, as in this research.[19]

The use of acid solutions for smear layer removal is frequently mentioned in the literature relative to permanent teeth.[7,9,11,14,15,18,28-30] A combination of solutions —such as EDTA, sodium hypochlorite, citric acid, and sodium hypochlorite—at various concentrations, times, and volumes of application, is considered the most effective, as these solutions combine agents with properties for demineralization and dissolution of organic matter.[17] The results obtained in this study agree with the findings in the literature on permanent teeth, since both samples irrigated with both the EDTA/sodium hypochlorite and citric acid/sodium hypochlorite combinations were shown to be effective for removing the smear layer and exposing the tubules.

In primary teeth, few studies have been developed that emphasize smear layer removal. Irrigant solutions such as sodium hypochlorite associated with oxygenated water, citric acid, and EDTA, in different protocols, are among those used.[19,31-33]

To verify the adaptation of filling paste (zinc oxide and eugenol) to the root canal walls, a study was developed in primary teeth using various irrigant solutions. Although adaptation was deficient in all the groups analyzed, the filling material penetrated into the dentinal tubules of the canals irrigated with EDTA.[31] Based on the literature on permanent teeth and on the results achieved in the present study, this suggests that the tubules had been exposed by the action of EDTA.

Regarding citric acid, the results of this study were similar to those found in the literature. Even with shorter application times (15 and 30 seconds) than those used in this study, it was reported that 6% citric acid promoted smear layer removal.[19] Another study, using the combination of 1% sodium hypochlorite and 10% citric acid, also verified smear layer removal and opening of tubules.[33] In an evaluation of smear layer removal in the pulp chamber, no differences were observed in the degree of tubule exposure when comparing citric acid solutions at 6%, 8%, and 10% associated with 1% sodium hypochlorite. Nevertheless, the authors suggested the use of the 6% solution for irrigating primary teeth, since the more concentrated solutions caused destruction of the intertubular dentin.[32]

No significant differences were observed between the irrigation regimes using ETDA or citric acid. This result does not agree with the findings of some studies indicating that sodium hypochlorite allied to EDTA is the most effective form of smear layer removal.[7,14] One study verified that final irrigation with EDTA allows a higher incidence of obturating the main canal ramifications of permanent teeth than the application of citric acid. That study, however, used a lower concentration of the latter solution (3%), which, among other methodological differences, could be responsible for the difference found between the results.[34] Other studies observed no significant differences between the 2 solutions.[15,16] The characteristics of primary teeth should be considered and discussed properly. The differences in volume and action time are also factors capable of influencing the results, however, they are difficult to measure,due to the various protocols adopted in the experiments.

CLINICAL RELEVANCE OF ENDODONTIC SMEAR LAYER REMOVAL IN PRIMARY TEETH

Although the results observed in studies developed in vitro cannot be immediately transferred to the clinic, they help allow a critical analysis to be made of the techniques clinically adopted. With this study, a treatment is suggested that may possibly be helpful in biomechanical preparation of primary tooth root canals through smear layer removal. The smear layer may contain bacteria in its composition and make it difficult to disinfect root canals. Once this study demonstrated the efficacy of EDTA and citric acid for smear layer removal in vitro, the possibilities for their clinical application (considering advantages and disadvantages) should be discussed and carefully considered in the primary dentition.

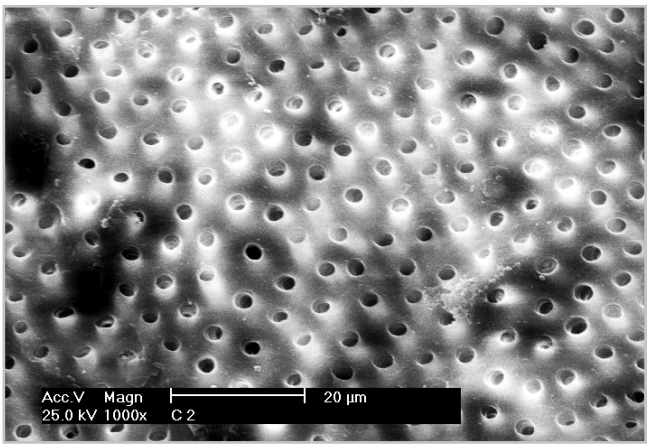

Figure 5. Photomicrograph of Group 3 showing open dentinal tubules (score 0).

First, it is important to analyze the advantages of smear layer removal. The smear layer is formed as a result of root canal debridement. It is composed of dentin scrapings associated with the organic contents present in the root canal at the time of biomechanical preparation, including blood, necrotic remains, and microorganisms.[6,17,25,35] Perhaps it contains viable bacteria and obliterates the dentinal tubules. If the micro-organisms have already invaded the tubules, the presence of the smear layer might protect them from the action of medications, such as sodium hypochlorite or calciumhydroxide. In permanent teeth, smear layer removal increases the diffusion of ions from the calcium hydroxide paste into the root canals.[36] When observing the canal systems of primary teeth, it should be noted that, in addition to obstructing the tubules, the smear layer also obstructs the entry to the ramifications of the main canal in which there could be necrotic and contaminated contents that manual instruments are incapable of reaching. Therefore, smear layer removal means facilitating access of these medications to all the confined areas of the primary tooth root canal, as has been verified in permanent teeth.[5,11]

Obviously, the smear layer is not completely impermeable; therefore, the medications can act even through this structure. If it is not impermeable, however, does the smear layer not "bury" the bacteria inside the tubules, and could those that remain viable be responsible for failure of the endodontic treatment? In an in vivo study that tested the antimicrobial effects of different irrigation regimes with sodium hypochlorite, either followed by a solution to remove the smear layer or not, it

was verified that the best results were obtained with the use of demineralizing solutions.[37] The authors suggested that the presence of the smear layer impeded the action of the sodium hypochlorite in the confined areas of the root canal, which was later reaffirmed by other studies.[35,38]

On the other hand, there is a very important aspect to consider: biocompatibility. Studies that evaluated the cytotoxicity and irritant potential of EDTA and citric acid solutions demonstrated that both cause tissue reactions, to a greater or lesser degree, and that citric acid presented lower cytotoxicity and greater biocompatibility.[39,40] The clinical use of EDTA in permanent teeth seems not to cause postoperative reactions.[38] One must consider, however, that primary teeth that have already started rhizolysis present a greater area of contact between the medications used in the root canal and the periapical tissues. Therefore, preference should be given to protocols that diminish the risk of tissue damage, such as citric acid. On the other hand, in the protocols suggested by this experiment, the solutions remain inside the canal for a short time and, as only a small quantity of solution is placed inside the canal, the risk of injection beyond the foramen is reduced. Furthermore, EDTA presents an irritant potential similar to that of sodium hypochlorite, a solution already accepted for irrigating primary teeth.

This study opens a range of issues for subsequent research. It is relevant to confirm, by means of laboratory and clinical studies, whether diffusion of medications through the dentin and disinfection of primary tooth root canals are really benefited, as occurs in permanent teeth. Therefore, some ideas can be discussed. A clinical study showed that isolated root canal mechanical preparation presented poor microbiological results. The use of intracanal dressing with calcium hydroxide after root canal mechanical preparation greatly increased the elimination of micro-organisms.[41] Since the smear layer removal increases the diffusion of ions from calcium hydroxide into the dentinal tubules,[36] it can be useful in cases of necrotic pulp and periapical lesions, where micro-organisms organized as biofilms on the external root surface (extraradicular infection) were detected in primary teeth.[42] Also, calcium hydroxide has a good tissue response[43] and antimicrobial activity against bacterial strains commonly found in endodontic infections of primary teeth.[44]

Biocompatibility studies, simulating clinical situations found in pediatric dentistry, such as primary teeth with rhizolysis and the presence of the permanent tooth bud, arouse scientific curiosity regarding the use of decalcifying substances inside the root canal and their reflectionon the permanent tooth bud.

CONCLUSIONS

Based on this study's results, the following conclusions can be made:

1. Biomechanical preparation of primary tooth root canals with 1% sodium hypochlorite causes smear layer formation.
2. It is possible to remove the smear layer with the use of the demineralizing irrigant solutions here tested: 17% ethylenediaminetetraacetic acid and 6% citric acid.
3. There is no statistically significant difference between 17% EDTA solution and the 6% citric solution regarding smear layer removal efficiency.

REFERENCES

1. Rifkin A. A simple, effective, safe technique for the root canal treatment of abscessed primary teeth. J Dent Child 1980;47:435-41.
2. Rosendahl R, Weinert-Grodd A. Root canal treatment of primary molars with infected pulps using calcium hydroxide as a root canal filling. J Clin Pediatr Dent 1995;19:255-8.
3. Hobson P. Pulp treatment of deciduous teeth. 1. Factors affecting diagnosis and treatment. Br Dent J 1970;128:232-8.
4. Oguntebi BR. Dentine tubule infection and endodontic therapy implications. Int Endod J 1994;27: 218-22.
5. Goldberg F, Abramovich A. Analysis of the effect of EDTAC on the dentinal walls of the root canal. J Endod 1977;3:101-5.
6. Goldman M, Goldman LB, Cavaleri R, Bogis J, Lin PS. The efficacy of several endodontic irrigating solutions—A scanning electron microscopic study: Part 2. J Endod 1982;8:487-92.
7. Yamada RS, Armas A, Goldman M, Lin PS. A scanning electron microscopic comparison of a high volume final flush with several irrigating solutions: Part 3. J Endod 1983;9:137-42.
8. Calas P, Rochd T, Druilhet P, Azais JM. In vitro adhesion of two strains of *Prevotella nigrescens* to the dentin of the root canal: The part played by different irrigation solutions. J Endod 1998;24:112-5.
9. Calas P, Rochd T, Michel G. In vitro attachment of *Streptococcus sanguis* to the dentin of the root canal. J Endod 1994;20:71-4.
10. Fogel HM, Pashley DH. Dentin permeability: Effects of endodontic procedures on root slabs. J Endod 1990;16:442-5.
11. Garberoglio R, Becce C. Smear layer removal by root canal irrigants: A comparative scanning electron microscopic study. Oral Surg Oral Med Oral Pathol 1994;78:359-67.
12. Haznedaroglu F. Efficacy of various concentrations of citric acid at different pH values for smear layer removal. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:340-4.

13. Hottel TL, el-Refai NY, Jones JJ. A comparison of the effects of three chelating agents on the root canals of extracted human teeth. J Endod 1999; 25:716-7.
14. Liolios E, Economides N, Parissis-Messimeris S, Boutsioukis A. The effectiveness of three irrigating solutions on root canal cleaning after hand and mechanical preparation. Int Endod J 1997;30:51-7.
15. Scelza MF, Antoniazzi JH, Scelza P. Efficacy of final irrigation: A scanning electron microscopic evaluation. J Endod 2000;26:355-8.
16. Scelza MF, Teixeira AM, Scelza P. Decalcifying effect of EDTA-T, 10% citric acid, and 17% EDTA on root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:234-6.
17. Sen BH, Wesselink PR, Turkun M. The smear layer: A phenomenon in root canal therapy. Int Endod J 1995;28:141-8.
18. Takeda FH, Harashima T, Kimura Y, Matsumoto K. A comparative study of the removal of smear layer by three endodontic irrigants and two types of laser. Int Endod J 1999;32:32-9.
19. Salama FS, Abdelmegid FY. Six percent citric acid better than hydrogen peroxide in removing smear layer: An in vitro pilot study. Pediatr Dent 1994; 16:424-6.
20. Rome WJ, Doran JE, Walker WA III. The effectiveness of glyoxide and sodium hypochlorite in preventing smear layer formation. J Endod 1985;11:281-8.
21. Brannstrom M. Smear layer: Pathological and treatment considerations. Oper Dent Suppl 1984;3: 35-42.
22. Moodnik RM, Dorn SO, Feldman MJ, Levey M, Borden BG. Efficacy of biomechanical instrumentation: A scanning electron microscopic study. J Endod 1976;2:261-6.
23. Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. J Endod 1992;18:605-12.
24. Ciucchi B, Khettabi M, Holz J. The effectiveness of different endodontic irrigation procedures on the removal of the smear layer: A scanning electron microscopic study. Int Endod J 1989;22:21-8.
25. Dautel-Morazin A, Vulcain JM, Bonnaure-Mallet M. An ultrastructural study of the smear layer: Comparative aspects using secondary electron image and backscattered electron image. J Endod 1994; 20:531-4.
26. Franchi M, Eppinger F, Filippini GF, Montanari G. NaOCl and EDTA irrigating solutions for endodontics: SEM findings. Bull Group Int Rech Sci Stomatol Odontol 1992;35:93-7.
27. Tatsuta CT, Morgan LA, Baumgartner JC, Adey JD. Effect of calcium hydroxide and four irrigation regimens on instrumented and uninstrumented canal wall topography. J Endod 1999;25:93-8.
28. Baumgartner JC, Brown CM, Mader CL, Peters DD, Shulman JD. A scanning electron microscopic evaluation of root canal debridement using saline, sodium hypochlorite, and citric acid. J Endod 1984; 10:525-31.
29. Dotto SR, Travassos RM, de Oliveira EP, Machado ME, Martins JL. Evaluation of ethylenediaminetetraacetic acid (EDTA) solution and gel for smear layer removal. Aust Endod J 2007;33:62-5.
30. Perez-Heredia M, Ferrer-Luque CM, Gonzalez-Rodriguez MP. The effectiveness of different acid irrigating solutions in root canal cleaning after hand and rotary instrumentation. J Endod 2006;32: 993-7.
31. Alacam A. The effect of various irrigants on the adaptation of paste filling in primary teeth. J Clin Pediatr Dent 1992;16:243-6.
32. Gotze Gda R, Cunha CB, Primo LS, Maia LC. Effect of the sodium hypochlorite and citric acid association on smear layer removal of primary molars. Braz Oral Res 2005;19:261-6.
33. Primo LS, Chevitarese O, Guedes-Pinto AC. Efficacy of irrigating solutions in removing radicular smear layer from anterior primary teeth [abstract]. J Dent Res 2002;81:411.
34. Holland R, Silva ACF, Bazaglia AM, Barros VCL, Magro VM. Influence of use of descalcificator solutions in obturation of radicular canals system. Rev Bras Odontol 1988;45:16-22.
35. Czonstkowsky M, Wilson EG, Holstein FA. The smear layer in endodontics. Dent Clin North Am 1990;34:13-25.
36. Foster KH, Kulild JC, Weller RN. Effect of smear layer removal on the diffusion of calcium hydroxide through radicular dentin. J Endod 1993;19:136-40.
37. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985;18:35-40.
38. Yoshida T, Shibata T, Shinohara T, Gomyo S, Sekine I. Clinical evaluation of the efficacy of EDTA solution as an endodontic irrigant. J Endod 1995;21: 592-3.
39. Malheiros CF, Marques MM, Gavini G. In vitro evaluation of the cytotoxic effects of acid solutions used as canal irrigants. J Endod 2005;31:746-8.
40. Sousa SM, Bramante CM, Taga EM. Biocompatibility of EDTA, EGTA, and citric acid. Braz Dent J 2005;16:3-8.
41. Faria G, Nelson-Filho P, Freitas AC, Assed A, Ito IY. Antibacterial effect of root canal preparation and calcium hydroxide paste (Calen) intracanal dressing in primary teeth with apical periodontitis. J Appl Oral Sci 2005;13:351-5.

42. Rocha CT, Rossi MA, Leonardo MR, Rocha LB, Nelson-Filho P, Silva LA. Biofilm on the apical region of roots in primary teeth with vital and necrotic pulps with or without radiographically evident apical pathosis. Int Endod J 2008;41:664-9.
43. Silva LAB, Leonardo MR, Oliveira DSB, et al. Histopathological evaluation of root canal filling materials for primary teeth. Braz Dent J 2010;21: 38-45.
44. Queiroz AM, Nelson-Filho P, Silva LAB, Assed S, Silva RAB, Ito IY. Antibacterial activity of root canal filling materials for primary teeth: Zinc oxide and eugenol cement, Calen paste thickened with zinc oxide, Sealapex, and EndoREZ. Braz Dent J 2009;20:290-6.