Comparison of removal of endodontic smear layer using ethylene glycol bis (beta-amino ethyl ether)-N, N, N', N'-tetraacetic acid and citric acid in primary teeth: A scanning electron microscopic study

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Abstract

Background: Root canal irrigants are considered momentous in their tissue dissolving property, eliminating microorganisms, and removing smear layer. The present study was aimed to compare the removal of endodontic smear layer using ethylene glycol bis (beta-amino ethyl ether)-N, N, N', N'-tetraacetic acid (EGTA) and citric acid solutions with saline as a control in primary anterior teeth. **Materials and Methods:** Thirty primary anterior teeth were chosen for the study. The teeth were distributed into three groups having ten teeth each. Following instrumentation, root canals of the first group were treated with 17% EGTA and the second group with 6% citric acid. Only saline was used as an irrigant for the control group. Then, the teeth were subjected to scanning electron microscopy (SEM) study. The scale given by Rome *et al.* for the smear layer removal was used in the present study. **Results:** The pictures from the SEM showed that among the tested irrigants, 17% EGTA + 5% sodium hypochlorite (NaOCl) group showed the best results when compared to other groups. **Conclusion:** The results advocate that the sequential irrigation of the pulp canal walls with 17% EGTA followed by 5% NaOCl produced efficacious and smear-free root canal walls.

Keywords: Citric acid, ethylene glycol bis (beta-amino ethyl ether)-N, N, N', N'-tetraacetic acid, primary teeth, smear layer

Introduction

Sustenance of deciduous dentition is fundamental for amicable occlusal development, preservation of arch length, optimal function of chewing, speech and maintenance of the healthy oral environment.[1] Conservation of the tooth is the primary aim of an endodontic treatment.[2]

Biomechanical preparation, irrigation and disinfection, and obturation are the three essential established phases in the endodontic treatment known as "ENDODONTIC TRIAD."[3] Smear layer is both organic and inorganic in composition. The inorganic material is composed of tooth structure and some nonspecific inorganic contaminants. The organic components may be formed of heated coagulated proteins, necrotic or viable pulp tissue, and odontoblastic processes plus saliva, blood cells, and microorganisms.[4] Whether it is beneficial or detrimental to a successful root canal therapy is still controversial. The smear layer may be favorable as it reduces the dentin permeability and prevents or decreases the bacterial incursion into the dentinal tubules. Conversely, the smear layer may also be considered detrimental because it prevents the infiltration of irrigants, medications, and filling materials into the dentinal tubules and may obstruct their contact with the canal wall.[5]

The simple act of irrigation causes the removal of smear layer and loose, necrotic, contaminated materials are washed off. They are unintentionally pushed deeper into the canal and apical tissues thus compromising the periapical tissue and permanent bud.[6] Preferably, the irrigants properties should comprise of antimicrobial and tissue-dissolution actions, lubrication, demineralization, and the ability to remove debris and the smear layer.[7] As, up till now, no single irrigant has been able to dissolve the organic pulpal material and demineralize the inorganic calcified portion of the pulp canal wall, hence an assortment of irrigants in

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different combinations has been suggested to accomplish these goals. It is established that irrigating the root canals with 10 ml of 17% ethylenediaminetetraacetic acid(EDTA) followed by 10 ml of 5% sodium hypochlorite (NaOCl) is the most effective method to remove the smear layer. EDTA chelates with Ca^{2+} and other divalent cations, demineralizes dentin, and removes the inorganic constituents of the smear layer but it also causes erosion. Yet another chelator ethylene glycol bis (beta-amino ethyl ether)-N, N, N', N'-tetraacetic acid (EGTA) is reported to bind Ca^{2+} more specifically without inducing erosion and thereby efficiently removes the inorganic component of the smear layer.[5]

The effectiveness of citric acid to remove smear layer has been demonstrated by Salama and Abdelmegid. In addition to removing smear layer, citric acid is a powerful antimicrobial agent.[4]

The present study is conducted with the aim to assess the capacity of various irrigants to remove the smear layer in deciduous teeth root canals following hand instrumentation.

Materials and Methods

Thirty primary anterior teeth were considered for this study [Table 1].

Primary anterior teeth with minimum two-third of root were chosen for the study. Teeth were stored in 0.9% saline solution. Dehydration and heat generation were avoided by constantly irrigating with saline solution.

The crowns of the teeth were cut at the horizontal; cut was made at cementoenamel junction. The root canals were gradually enlarged with K-files to size 45. The biomechanical preparation was done using K-files. Irrigation during cleaning and shaping was done using 3 ml of saline solution. After instrumentation, irrigation in the root canals of the first group was done with 10 ml of 17% EGTA (Sigma, St. Louis, MO, USA) and subsequently with 10 ml of 5% NaOCl. The second group was irrigated with 10 ml of 6% citric acid. The teeth in the control group were irrigated with only 10 ml of 0.9% saline.

Table 1: Distribution of samples of each group using three irrigating solutions for smear layer removal (30 teeth)

Group	Section	*n*
Group I: 17% EGTA	Apical	10
	Middle	10
	Coronal	10
	Total	30
Group II: 6% citric acid	Apical	10
	Middle	10
	Coronal	10
	Total	30
Group III: 0.9% saline	Apical	10
	Middle	10
	Coronal	10
	Total	30

EGTA: Ethylene glycol bis (beta-amino ethyl ether)-N, N, N', N'-tetraacetic acid

Preparation of the irrigation solution

- About 17% EGTA solution-17 g of EGTA was dissolved in 100 ml of distilled water at pH 7.5 by addition of NaOH (laboratory preparation)
- About 6% citric acid solution-6 g of citric acid was dissolved in 100 ml of distilled water.

Splitting of samples

All the roots were sectioned longitudinally and processed for scanning electron microscopy evaluation.

Scanning electron microscopy

- Representative photographs from cervical, middle, and apical root third were taken for all the samples at magnification ×4000
- Images were scored according to the scoring criteria, a modification of scoring method of Kumar and Anita.[2]

Score

- 0 - No smear layer, all dentinal tubules open and no erosion of tubules
- 1 - No smear layer, all dentinal tubules open and erosion of tubules
- 2 - Minimum smear layer >50% dentinal tubules visible
- 3- Moderate smear layer; <50% of dentinal tubules open
- 4 - Heavy smear layer; outline of dentinal tubules obliterated.

Results

Two-way analysis of variance was applied for statistically analyzing the differences in the three groups, subsequently *post hoc* Bonferroni's multiple comparisons was done for intercomparison between individual groups for differences. All testing were done using two-sided tests with alpha 0.05.

Group I was superior in the removal of smear layer in comparison with the other two groups [Figure 1]. Alteration of dentinal tubules was noted in Group II [Figure 2]. Group III did not remove the smear layer at all [Table 2 and Figure 3].

The intragroup comparison of efficacy of root canal irrigants within the cervical, middle, apical thirds showed that the cervical third showed the best smear layer removal in EGTA group, while the middle third showed the most excellent smear layer removal in the citric acid group [Table 3 and Graph 1].

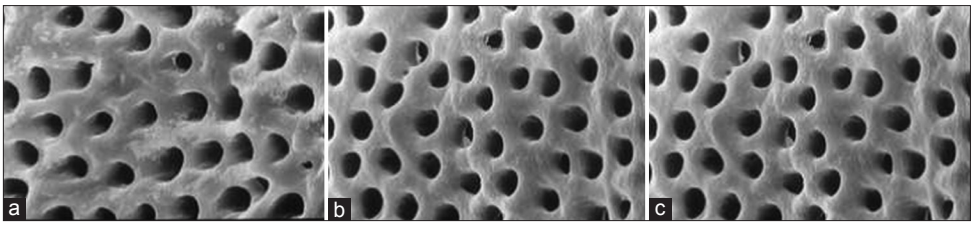


Figure 1: (a) Apical third of Group I, (b) middle third of Group I, and (c) coronal third of Group I

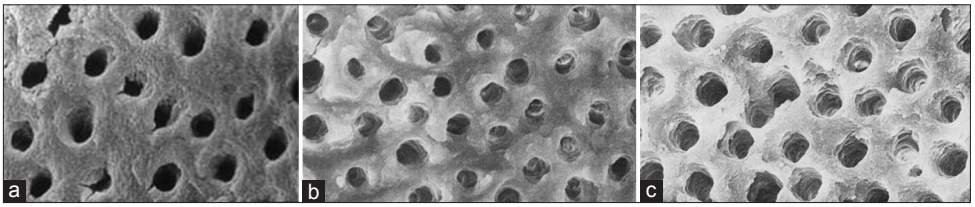


Figure 2: (a) Apical third of Group II, (b) middle third of Group II, and (c) coronal third of Group II

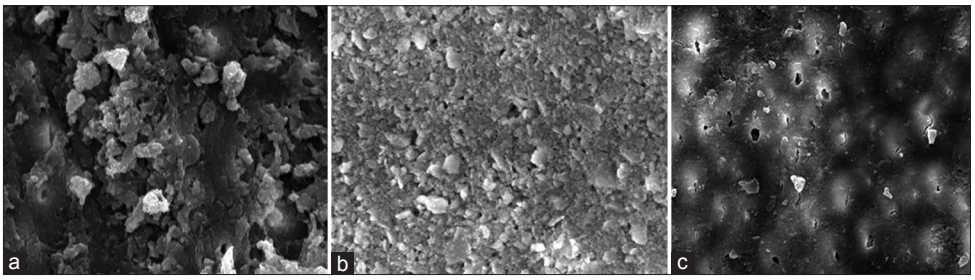
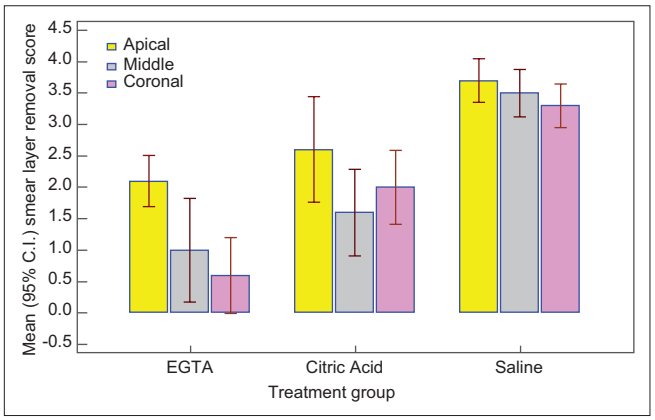


Figure 3: (a) Apical third of Group III, (b) middle third of Group III, and (c) coronal third of Group III

Table 2: Intergroup comparison for smear layer removal between the three tested irrigants

Intergroup comparison	Mean difference	SE	*P*[a]	95% CI[a]
Group I: 17% versus EGTA				
Saline	−2.2667	0.2124	<0.0001	−2.7859-1.7475
Citric acid	−0.8333	0.2124	0.0005	−1.3525-0.3141
Group II: 6% citric versus acid				
Saline	−1.4333	0.2124	<0.0001	−1.9525-0.9141
EGTA	0.8333	0.2124	0.0005	0.3141-1.3525
Group III: 0.9% versus saline				
EGTA	2.2667	0.2124	<0.0001	1.7475-2.7859
Citric acid	1.4333	0.2124	<0.0001	0.9141-1.9525

[a]Bonferroni corrected. EGTA: Ethylene glycol bis (beta-amino ethyl ether)-N, N, N', N'-tetraacetic acid; SE: Standard error; CI: Confidence interval



Graph 1: Intragroup comparison between different sections of the teeth (apical, middle, and coronal third) for smear layer removal

Discussion

Various chemicals, organic acids, ultrasonic devices, and lasers have been used in the removal of smear layer.[8] Most commonly used irrigating solutions are NaOCl in varying concentrations of 1–5.25%, 17% EDTA, citric acid in concentrations varying from 1% to 50%, MTAD and polyacrylic acid in 5, 10 and 20% concentrations.[1]

Most of the studies using various irrigating solutions to remove smear layer have been conducted on permanent teeth but their effect on primary teeth has not been studied.[4] The differences among the dentin substrata of primary and permanent teeth should be considered before choosing a cleanser in the pulpal therapy of primary teeth.[1] The dentinal

Table 3: Intragroup comparison between different sections of the teeth (apical, middle, and coronal third) for smear layer removal

Group	Section	*n*	Mean	SE	95% CI
Group I: 17% EGTA	Apical	10	2.1000	0.2601	1.5825-2.6175
	Middle	10	1.0000	0.2601	0.4825-1.5175
	Coronal	10	0.6000	0.2601	0.08247-1.1175
	Total	30	1.2333	0.1502	0.9345-1.5321
Group II: 6% citric acid	Apical	10	2.6000	0.2601	2.0825-3.1175
	Middle	10	1.6000	0.2601	1.0825-2.1175
	Coronal	10	2.0000	0.2601	1.4825-2.5175
	Total	30	2.0667	0.1502	1.7679-2.3655
Group III: 0.9% saline	Apical	10	3.7000	0.2601	3.1825-4.2175
	Middle	10	3.5000	0.2601	2.9825-4.0175
	Coronal	10	3.3000	0.2601	2.7825-3.8175
	Total	30	3.5000	0.1502	3.2012-3.7988

EGTA: Ethylene glycol bis (beta-amino ethyl ether)-N, N, N', N'-tetraacetic acid; SE: Standard error; CI: Confidence interval

permeability in primary teeth is less than permanent teeth due to a reduced amount of mineralization of primary tooth dentin.[9]

The morphology of the root canals of primary teeth as well as the microbiology of the infected teeth are significant barriers to adequate biomechanical cleaning.[4] Few of the zones of primary teeth such as accessory canals, ramifications, and dentinal tubules are unapproachable for debridement. For that reason, the role of auxiliary solutions which promote disinfection of these areas is vital. Largely because dentinal tubules of infected primary teeth can harbor microorganisms within them just as the permanent teeth.[1] Since many of the root canal ramifications cannot be reached mechanically, copious irrigation is important during cleaning and shaping.[4]

Hence, the present *in vitro* study was carried out to evaluate the effect of 17% EGTA, 6% citric acid, and 0.9% saline as irrigating solutions for evaluating smear layer removal in primary teeth.

Keeping in mind the inability of a single irrigant to dissolve the organic pulpal material and predentin in addition to demineralize the organic calcified portion of the pulp canal wall, a blend of various irrigants have been suggested to accomplish these goals.[10]

Group I (17% EGTA followed by 5% NaOCl) showed the best efficacy in removing smear layer when compared to 6% citric acid (Group II) and Saline (Group III) at all the three sections (apical, middle, and coronal third). No signs of erosion of dentinal structures were noted.

This is in accordance with the studies conducted by Torabinejad *et al*. (2003) who evaluated the effects of EGTA in contrast with EDTA on permanent teeth. EGTA was not as effective in the apical third when compared to coronal and middle third of the teeth.[11] Similar study was conducted by Viswanath *et al*. who compared EGTA and EDTA on permanent teeth and observed that EGTA was more efficient in removing smear layer.[5]

The lower erosive potential of EGTA on root canal dentin has led to its introduction as an endodontic irrigant in various *in-vitro* studies. Its property for removal of smear layer could be attributed to the chelating property of EGTA that dissolves the inorganic content of the root canal by reacting with calcium ions and forms soluble chelates.[11]

Six percent citric acid showed erosion of dentinal tubules. This is in accordance with studies done by Balto *et al*. (2015).[12]

The inability of the saline group in the removal of smear layer has been proved by the highest score recorded in that group. This is supported by the earlier studies done by Wayman *et al*.(1979), Yamada *et al*.(1983), Berg *et al*. (1986), and Baumgartner and Mader (1987) in permanent teeth and Salama and Abdelmegid in primary teeth.[4]

Intragroup comparison between different sections of the teeth demonstrates that coronal third of Group I showed the best smear layer removal when weighed against the middle and apical section. This is in accordance and supports the study conducted by Hariharan *et al*. (2010) who showed that coronal section of the teeth showed the best smear layer removal.[13]

Conclusion

The results from the present study suggest that the sequential irrigation of the pulp canal walls with 17% EGTA followed by 5% NaOCl produced efficacious and smear free root canal walls. Further investigations regarding its *in vivo* use are advocated to establish its use in primary teeth.

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Conflicts of interest
There are no conflicts of interest.

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