



Effect of EDTA and Sodium Gluconate on Smear Layer removal and dentin decalcification- An *in-vitro* scanning electron microscopic evaluation

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ABSTRACT: The aim of this study is to evaluate the ability of sodium gluconate along with 17% EDTA when used as a final irrigant in smear layer removal and dentin decalcification.

Materials and methodology

Forty single-rooted mandibular premolars were collected and prepared for the study. Following preparation, the specimens were exposed to the test solutions divided into 4 groups namely,

Group A – Five ml of 17% EDTA used as a final irrigant (n=10)

Group B – Five ml of 16% sodium gluconate followed by five ml of 17% EDTA used as a final irrigant. (n =10)

Group C – Five ml of 16% sodium gluconate used as a final irrigant (n=10)

Group D– Saline (n=10)

Then the specimens were sectioned longitudinally by placing longitudinal grooves on the buccal and lingual aspect with a double sided diamond disc and split into two halves with a chisel and mallet. One-half of each sample with an adequate canal portion was selected and subjected to scanning electron microscopic (SEM) analysis for the evaluation of smear layer removal and dentinal erosion.

Results

The smear layer removal ability of groups A , B and C was effective in the coronal and middle thirds of the root canals . The dentinal erosion of group A , B , C in apical thirds is lesser than the coronal and middle thirds.

Conclusion

The study concludes that the smear layer removal capability of sodium gluconate alone was more effective than the EDTA and sodium gluconate

combined. The sodium gluconate along with EDTA combined showed more dentinal erosion than sodium gluconate alone used as an irrigant.

I. INTRODUCTION

The successful outcome of root canal treatment significantly depends on the meticulous cleaning and shaping of the root canal system. The instrumentation of the root canal leaves a smear layer covering the dentinal walls. This layer can form two zones: the first, 1–2 μ m thick, made up of organic matter and dentine particles; the second, extending into dentinal tubules to a depth of 40 μ m (smear plugs) is formed largely of dentine chips⁽¹⁾. It is known that the smear layer may harbour bacteria, preventing the canal from being disinfected. In addition, it has been demonstrated that the removal of this layer promotes dentine permeability, enhancing diffusion and the action of intracanal medication, allowing and producing greater penetration of filling material into lateral canals and dentinal tubules. Unfortunately, no irrigating solution is capable of acting simultaneously on the organic and inorganic elements of the smear layer⁽²⁾.

The smear layer produced during root canal instrumentation consists of organic and inorganic debris that may interfere with effective disinfection and sealing of the root canal system. Various irrigants, including sodium hypochlorite, organic acids, and chelating agents, have been advocated for its removal. Among chelators, ethylenediaminetetraacetic acid (EDTA), particularly in its disodium salt form, is the most widely used agent in contemporary endodontic practice⁽³⁾.



The chelating efficacy of EDTA is dependent on multiple variables such as its concentration, duration of application, canal length, and dentin hardness. EDTA acts by indiscriminately dissolving the inorganic components of the smear layer as well as the underlying dentin, leading to exposure of collagen fibers. When used sequentially or simultaneously with sodium hypochlorite, EDTA has been shown to cause dentinal erosion and a reduction in dentin microhardness. These alterations include widening of dentinal tubules, softening of dentin, and collagen degradation, which may adversely affect the adaptation and sealing of obturating materials. Despite these drawbacks, alternating irrigation with EDTA and sodium hypochlorite remains the most effective protocol for smear layer removal.

Evidence suggests that 5.25% sodium hypochlorite is the irrigant of choice during instrumentation, while a final rinse of 17% EDTA followed by sodium hypochlorite yields optimal smear layer removal⁽⁴⁾. However, the aggressive nature of EDTA has prompted research into alternative chelating agents with controlled demineralizing properties.

Sodium gluconate, a salt of gluconic acid derived from *Zea mays* through microbial fermentation using *Aspergillus niger*, has emerged as a potential chelating agent. It is a water-soluble, biodegradable, non-toxic, and non-corrosive compound with a high affinity for divalent and trivalent metal ions. Its chelating efficiency under alkaline conditions has been reported to be comparable to that of EDTA⁽⁵⁾. Although widely used in pharmaceutical and industrial applications, its role in endodontic irrigation remains largely unexplored. Therefore, the present study aimed to evaluate the effectiveness of sodium gluconate, alone and in combination with 17% EDTA, as a final irrigant for smear layer removal and dentinal decalcification.

II. MATERIALS AND METHODOLOGY

Preparation of sodium gluconate solution

Sodium gluconate solution was prepared at a concentration of 16% by dissolving 16 g of sodium gluconate powder in 100 ml of sterile water stabilized to a pH of 9 with 1 ml 0.1 N NaOH⁽⁶⁾.

Sample preparation

Forty extracted human mandibular premolars with single straight canals and fully formed apices were selected for this in vitro study. Teeth were extracted for orthodontic or periodontal reasons. Specimens exhibiting caries, root resorption, canal calcification, severe curvature, or previous endodontic treatment were excluded.

All teeth were decoronated using a diamond disc under slow-speed rotation to obtain a standardized root length of 15 mm. Canal patency was established with size 10 and 15 K-files. Cleaning and shaping were performed using the NeoEndo-S rotary file system following the manufacturer's recommended sequence. Instrumentation was completed up to size 25 with a 0.06 taper.

Throughout canal preparation, irrigation was carried out using 5.25% sodium hypochlorite delivered via a side-vented needle placed 1 mm short of the working length to ensure adequate irrigant penetration while minimizing apical extrusion.

Following canal preparation, the specimens were randomly divided into 4 groups namely,

Group A – Five ml of 17% EDTA used as a final irrigant (n=10)

Group B – Five ml of 16% sodium gluconate followed by 5ml of 17% EDTA used as a final irrigant. (n=10)

Group C – Five ml of 16% sodium gluconate used as a final irrigant (n=10)

Group D – Saline (n=10)

Teeth sectioning The teeth samples were then sectioned longitudinally by placing two longitudinal grooves on the buccal and lingual aspect with a diamond disc and split into two halves with a chisel and mallet. One-half of each sample with an adequate canal portion was selected and subjected to scanning electron micro scope (SEM) analysis⁽⁷⁾.

Scanning electron microscope analysis

Following final irrigation protocols, the specimens were mounted on metallic stubs and sputter-coated with gold to enhance surface conductivity. Scanning electron microscopy (SEM) analysis was performed at magnifications of 1000× and 5000×. Images were obtained from the coronal, middle, and apical thirds of each specimen, yielding a total of six images per sample. Smear layer removal was assessed using the criteria described by Roedig et al.⁽⁸⁾, while dentinal erosion was evaluated based on the criteria proposed by Torabinejad et al.⁽⁹⁾. Image analysis was performed by a blinded examiner to eliminate observer bias. The median, first quartile (Q1) and third quartile (Q3) scores of all the specimens in the coronal, the middle, and the apical third were calculated and analysed statistically using SPSS software version 22.0 with Kruskal wallis test.

Roedig et al. criteria for evaluating smear layer removal:

1 - No smear layer, dentinal tubules open.

2 - Small amount of smear layer, some dentinal tubules open.



3 - Homogenous smear layer covering the root canal wall, only a few dentinal tubules open.

4 - Complete root canal wall covered by a homogenous smear layer, no open dentinal tubules.

5 - Heavy inhomogeneous smear layer covering the complete root canal wall.

Torabinajed et al. criteria for evaluating dentinal erosion:

1. No erosion–All tubules looked normal in appearance and size.

2. Moderate erosion – The periradicular dentine was eroded.

3. Severe erosion – The intertubular dentine was destroyed and tubules were connected with each other.

STATISTICAL ANALYSIS

The data obtained was subjected to analysis using SPSS software version 22.0. Following the conformation of non-parametric distribution of data, the data were analysed using Kruskal wallis test to compare the medians of four independent groups i.e., A, B ,C,D taking into consideration p<0.05 as statistically significant.

III. RESULTS

TABLE 1

Irrigation solution	Apical third	Middle third	Coronal third
	Median (IQR)	Median (IQR)	Median (IQR)
GROUP A	2 (1-2)	2(1-2)	2(1-2)
GROUP B	2(1-2)	1(1-2)	1(1-2)
GROUP C	2(1-2)	1(1-2)	1(1-2)
GROUP D	4(4-5)	4(4-4)	4(4-4)
p-Value	<0.001	<0.001	<0.001

TABLE 2

Irrigation solution	Apical third	Middle third	Coronal third
	Median (IQR)	Median (IQR)	Median (IQR)
GROUP A	2 (1-2)	2(2-2)	2(2-2)
GROUP B	2(1-2)	2(2-2)	2(2-2)
GROUP C	1(1-1)	1(1-1)	1(1-1)
GROUP D	0	0	0
p-Value	<0.001	<0.001	<0.001

IV. DISCUSSION

EDTA remains the most commonly used chelating agent in endodontics due to its strong affinity for calcium ions. As a hexadentate ligand, EDTA binds metal ions through four carboxylate and two amine groups, making it effective across a wide pH range. Mello et al.⁽¹⁰⁾ demonstrated that a three-minute application of EDTA effectively removes the smear layer from root canal walls. However, excessive use has been associated with dentinal erosion and weakening of root structure.

Fernandez et al.⁽¹¹⁾ reported that EDTA not only eliminates smear debris but also initiates surface erosion by excessive demineralization and

The median and interquartile range values of the smear layer and those values of dentinal erosion of all the samples were listed in Table 1 and Table 2, respectively, and were analysed by Kruskal wallis test using SPSS software version 22.0.

KRUSKAL WALLIS TEST

Evaluation of smear layer removal

The smear layer removal ability of groups A , B and C was effective in the coronal and middle thirds of the root canals . In group A, B and C, the apical third showed a median score of 2. Group B showed a median score of 1 in middle and coronal thirds. Group D did not show any significant result.A statistically significant difference was observed between the groups (p<0.001).

Evaluation of dentinal erosion

The dentinal erosion in group A and B showed a median score of 2 in the apical third , the coronal and the middle thirds. Group C, a median score of 1 was observed in the apical third, the coronal and the middle thirds. Group D did not show any significant resultA statistically significant difference was observed between the groups (p<0.001).

widening of dentinal tubules. This aggressive action underscores the need for alternative chelating agents with controlled activity.

Sodium gluconate is a polyhydroxycarboxylic acid whose chelating potential increases under alkaline conditions⁽¹²⁾. Its calcium-chelating mechanism primarily involves coordination through carboxylic oxygen atoms and α-hydroxyl groups⁽¹³⁾. Effective calcium chelation requires an alkaline environment, as high pH promotes deprotonation of hydroxyl groups, creating active anionic sites for metal binding⁽¹⁴⁾.

In the present study, sodium gluconate was used at a pH of 9, under which it selectively formed



calcium gluconate complexes predominantly through carboxyl group interactions. Abdelazim et al.⁽⁵⁾ reported that sodium gluconate selectively chelates calcium even in the presence of competing cations at mildly alkaline pH levels.

The findings of this study suggest that sodium gluconate is less aggressive than EDTA in chelating calcium from the hydroxyapatite matrix, resulting in reduced dentinal erosion while still achieving effective smear layer removal. The combination of sodium gluconate and EDTA enhanced smear layer removal but increased dentinal erosion, highlighting the dominant demineralizing action of EDTA.

V. CONCLUSION

The study concludes that the smear layer removal capability of sodium gluconate alone was more effective than the EDTA and sodium gluconate combined. The sodium gluconate along with EDTA combined showed more dentinal erosion than sodium gluconate alone. To overcome this, the concentration of EDTA may be reduced and used along with sodium gluconate as a final irrigant.

REFERENCES

- [1]. Teixeira CS, Felipe MC, Felipe WT. The effect of application time of EDTA and NaOCl on intracanal smear layer removal: an SEM analysis. *International endodontic journal*. 2005 May;38(5):285-90.
- [2]. Gutiérrez JH, Herrera VR, Berg EH, Villena F, Jofré A. The risk of intentional dissolution of the smear layer after mechanical preparation of root canals. *Oral Surgery, Oral Medicine, Oral Pathology*. 1990 Jul 1;70(1):96-108.
- [3]. Şen BH, Ertürk Ö, Pişkin B. The effect of different concentrations of EDTA on instrumented root canal walls. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*. 2009 Oct 1;108(4):622-7.
- [4]. 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. *Journal of endodontics*. 1983 Apr 1;9(4):137-42.
- [5]. Abdelazim MH, Abdelazim AH. Effect of sodium gluconate on decreasing elevated nasal calcium and improving olfactory function post COVID-19 infection. *Am J Rhinol Allergy* 2022; 36(6):841–8.
- [6]. Karthikeyan HR, Rajakumaran A, Rajendran MR, Balaji L. Evaluation of Effect of Natural Extract Sodium Gluconate on Smear Layer and Dentine Decalcification Compared with EDTA—An In-vitro Study. *European Endodontic Journal*. 2023;8(4):274.
- [7]. Turk T, Kaval ME, Şen BH. Evaluation of the smear layer removal and erosive capacity of EDTA, boric acid, citric acid and desy clean solutions: an in vitro study. *BMC Oral Health*. 2015 Sep 3;15(1):104.
- [8]. Roedig T, Huelsmann M, Kahlmeier C. Comparison of root canal preparation with two rotary NiTi instruments: ProFile. 04 and GT Rotary. *International endodontic journal*. 2007 Jul;40(7):553-62.
- [9]. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, Kim J, Shabahang S. A new solution for the removal of the smear layer. *Journal of endodontics*. 2003 Mar 1;29(3):170-5.
- [10]. Mello I, Kammerer BA, Yoshimoto D, Macedo MC, Antoniazzi JH. Influence of final rinse technique on ability of ethylenediaminetetraacetic acid of removing smear layer. *Journal of endodontics*. 2010 Mar 1;36(3):512-4
- [11]. Fernández ML, Pérez GG, Villagómez MO, Villagómez GO, Báez TD, Lara GG. In vitro study of erosion caused by EDTA on root canal dentin. *Revista Odontológica Mexicana Órgano Oficial de la Facultad de Odontología UNAM*. 2012;16(1):8-13.
- [12]. Abbadi A, Gotlieb KF, Meiberg JB, Peters JA, Van Bekkum H. New Ca-sequestering materials. Based on the oxidation of the hydrolysis products of lactose. *Green Chemistry*. 1999;1(5):231-5.
- [13]. Phadungath C, Metzger LE. Effect of sodium gluconate on the solubility of calcium lactate. *Journal of dairy science*. 2011 Oct 1;94(10):4843-9.
- [14]. Hodge JE, Nelson EC, Moy BF. Chelates in agriculture, metal chelation by glucose-ammonia derivatives. *Journal of Agricultural and Food Chemistry*. 1963 Mar;11(2):126-9.
- [15]. Sawyer DT. Metal-gluconate complexes. *Chemical Reviews*. 1964 Dec 1;64(6):633-43.
- [16]. Sitashi P, Pan WH. Effect of ethylenediaminetetraacetic Acid (EDTA) gel on removing smear layer of root canal in vitro. *Chinese Medical Sciences Journal*. 2012 Oct 8;27(3):190-1.