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Pesquisa Científica

Comparative evaluation of smear layer removal efficacy using various final irrigation protocols: a SEM study

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Abstract

Objective: This study aimed to assess the efficacy of 0.2% Chitosan and 17% EDTA, with or without ultrasonic activation, in removing the smear layer (SL). Methods: Fifty bovine incisors were sectioned 17 mm from their apices, instrumented using ProTaper Universal instruments and divided into five groups (n=10): I- Distilled water + conventional irrigation (CI) (control), II- 17% EDTA + CI, III- 0.2% Chitosan + CI, IV- 17% EDTA + passive ultrasonic irrigation (PUI) and V- 0.2% Chitosan + PUI. The roots were split longitudinally and examined under a scanning electron microscope at 500x magnification to assess SL removal. SL presence was scored by three blinded examiners using a five-point scale. Data was analyzed using Kruskal-Wallis and Mann-Whitney U-tests ($p<0.05$). Results: The statistical analysis revealed significant differences in SL removal among groups ($p<0.05$). Group IV (17% EDTA + PUI) exhibited significantly higher removal efficacy than other groups ($p<0.05$). SL removal was highest in the cervical third compared to middle and apical thirds. Conclusions: 17% EDTA combined with PUI demonstrated superior SL removal.

KEYWORDS: Chelating Agents; Passive Ultrasonic Irrigation; Smear Layer.

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Introduction

Success in root canal treatment relies on the adoption of a therapeutic protocol that emphasizes rigorous sanitation processes[1]. The mechanical action of endodontic instruments on dentin walls generates an amorphous, irregular, and granular layer known as smear layer (SL)[2]. This layer comprises organic fragments of pulp tissue, necrotic remnants, irrigant solution, blood cells, microorganisms, and an inorganic component consisting of dentinal debris[3]. Removing the SL enables the neutralization of microbiota through the action of irrigating solutions and intracanal dressing, facilitates root canal sealer adhesion, reduces microleakage, and enhances the bond strength of fiberglass posts to root dentin[4].

Several solutions have been proposed for SL removal, including chelating and descaling agents[2,5,6]Q-MIX, and phytic acid in smear layer removal: A comparative scanning electron microscope study", "type" : "article-journal", "volume" : "8" }, "uris" : ["http://www.mendeley.com/documents/?uuid=371e895a-0eeb-4ea1-b60a-cf01e60e36e9"] }, { "id" : "ITEM-2", "itemData" : { "ISSN" : "1735-7497", "PMID" : "25031596", "abstract" : "INTRODUCTION Smear layer (SL. Ethylenediaminetetraacetic acid (EDTA) is the most used chelating agent[5]. However, despite its effectiveness, EDTA has been recognized as an environmental pollutant[7]S]-Ethylene Diamine Disuccinate ([S,S]-EDDS. As a result, some natural alternatives to EDTA, such as lactic acid, apple vinegar, citric acid, and chitosan have been investigated[2,6,8,9].

Chitosan is a natural polysaccharide derived from the N-deacetylation of chitin extracted from arthropod exoskeletons[9]. This substance offers biocompatibility, bioadhesion, biodegradability and is non-toxicity to the human body[8,9]. In Endodontics, chitosan has been studied for its antimicrobial effects and its use as a chelating agent has shown promising results in SL removal[8-11]. Kamble *et al.*[12] (2017) reported that 0.2%

chitosan solution exhibited superior SL removal compared to EDTA 17%, particularly in the apical third of the root canal.

It is known that chelating agents can be used with various protocols in root canal treatment, with ultrasonic agitation being an approach that enhances SL removal[3,13-15]. Ultrasonic agitation functions through acoustic microinterference and hydrodynamic cavitation, which facilitate the formation and implosion of vapor bubbles[16,17]. There is limited literature directly confronting chitosan with ultrasonic agitation in the removal of SL[8,9,14].

This study aimed to assess the efficacy of 0.2% Chitosan and 17% EDTA, with or without ultrasonic agitation, in terms of SL removal. The null hypothesis tested was that there would be no differences among the different final irrigation protocols regarding SL removal.

Methods

The present study protocol underwent review and approval by the Institutional Animal Care and Use Committee of the University of North Paraná, Londrina, Brazil (Protocol number 046-15).

Sample selection

Fifty bovine incisors were selected based on their anatomically similar size and shape of roots, with root canals measuring less than 1 mm in cervical diameter, as determined using a digital caliper (Mitutoyo, Tokyo, Japan). These teeth also had mature apices. They were stored in 0.05% chloramine solution (Pharm, Phloraceae, Cuiabá, MT, Brazil) at room temperature until use.

The teeth were decoronated below the cementoenamel junction utilizing a double-faced diamond disk (KG Sorensen, São Paulo, SP, Brazil) operated perpendicularly to their longitudinal axis to achieve standardized roots measuring 17 mm in length. Initially, a size 15 K-File (Dentsply Maillefer, Ballaigues, Switzerland) was

employed to confirm the patency of the canals. Subsequently, the anatomic diameter of all roots was standardized using a size 20 K-File (Dentsply Maillefer).

The root canal working length (RCWL) was established at 16 mm, and preflaring was conducted using a size 2 LA Axxess bur (35/.06) (SybronEndo Corporation, Orange, CA, USA), powered by Intramatic 2068 and Intramatic 181DBN (Kavo Ind. Com. Ltda., Joinville, SC, Brazil) motors operating at 5000 rpm and used until resistance to penetration was encountered. Root canal preparation (RCP) was carried out with the ProTaper Universal nickel-titanium system (Dentsply Maillefer) up to a size F5 instrument (50/.05). Each instrument was utilized for preparing only five root canals. Throughout RCP, the canals were irrigated with 2 mL of 2.5% sodium hypochlorite (NaOCl) solution (Pharm, Phloraceae). Following RCP, the apices of roots were sealed with flowable composite (Resin Opallis Flow; FGM, Joinville, SC, Brazil), the roots were dried using absorbent paper points (Dentsply Maillefer), and then randomly allocated into one control group (n=10), which received no final irrigant protocol, and four experimental groups (n=10). The experimental groups were defined based on the combination of the following factors: chelating solution (17% EDTA and 0.2% Chitosan) and final irrigant activation/delivery method [conventional irrigation (CI) and passive ultrasonic irrigation (PUI)] (Table 1).

TABLE 1 · Experimental groups distribution according to the chelating agent and irrigant activation/delivery method.

Group	Chelating agents	Irrigant activation/delivery method
1	Distilled water (control)	Conventional irrigation
2	17% EDTA	Conventional irrigation
3	0.2% Chitosan	Conventional irrigation
4	17% EDTA	Passive Ultrasonic irrigation
5	0.2% Chitosan	Passive Ultrasonic irrigation

The chelating agents utilized were prepared from analytical reagent-grade materials (Pharm, Phloraceae) using purified water obtained through a reverse osmosis system with ultraviolet light (Quimis, Diadema, SP, Brazil), ensuring electrical conductivity of <1 μS mm-2. The pH of the solutions was determined using a digital pH meter (Analion, Ribeirão Preto, SP, Brazil). The 0.2% Chitosan solution was prepared by dissolving 0.2 g of Chitosan (ACROS Organics Gell, Belgium; degree of deacetylation > 90%) in 100 mL of 1% acetic acid. The mixture was then agitated using a magnetic agitator for 2 hours[8,9].

Regarding the final irrigant activation/delivery method, in groups 2 and 3, 5 mL of non-activated chelating solutions were introduced into root canals using a 5 mL disposable syringe (Ultradent Products Inc, South Jordan, UT, USA) coupled with a 29-gauge (NaviTip; Ultradent Products Inc). The needle was inserted 1 mm short of RCWL without binding to the canal walls and left in place for 3 min.

In groups 4 and 5, 5 mL of chelating solutions were passively activated for 60 seconds using a Piezo-Electric MTS ultrasonic unit (Multitask Cart, Spartan Obtura, USA) along with an ultrasonic tip size 15 file (Satelec, Acteon, France). The ultrasonic tip was positioned 1 mm short of the RCWL without contacting the root canal walls, allowing it could vibrate freely. The ultrasonic unit was set to 40% power.

Lastly, the specimens underwent irrigated with 2 mL of 2.5% NaOCl (Pharm, Phloraceae), followed by a rinse of 10 mL of distilled water, and were subsequently dried with absorbent paper points (Dentsply Maillefer) and stored. All clinical procedures were conducted by a single operator (an endodontist with 10 years of experience).

Scanning electron microscope (SEM) analysis

Longitudinal grooves were carefully made on the buccal and lingual surfaces of each root using a carborundum disc (KG

Sorensen) ensuring the canals were not exposed. The final section of the samples was performed using a bi-bevelled chisel (Hu-Friedy Co, Chicago, IL, USA). The root side of the root with fewer irregularities, which most accurately represented the total root canal length, was selected. Subsequently, the samples were dehydrated in an ascendant alcohol battery (50%, 60%, 70%, 80%, 96%, and 100%; MercK, Darmstadt, Germany), followed by carbon dioxide drying using Autosamdri 815 (Tousimis Research Corporation, Maryland, USA). A 30 nm layer of gold was applied to the samples using a vacuum metallization apparatus (Denton Vacuum Innovations, Moorestown, USA) under the following conditions: pressure of 0.01 mbar, current of 40 mA, distance of 50 mm, and coverage time of 110 s.

Subsequently, the SEM analysis (Jeol JSM 6610, Thermo Scientific NSS Spectral Imaging, Tokyo, Japan), with an excitatory wavelength of 448 nm, was conducted. Photomicrographs at x500 magnification were captured from the apical, middle, and cervical portions of the root canal, positioned 3, 9, and 15 mm short of the root apex, respectively. Each photomicrograph was evaluated by three blinded and calibrated examiners. Scores ranging from 1 to 5 were assigned based on presence of the SL. Score 1 indicated SL covering the entire surface. Score 2 denoted SL partially covering the surface with few visible tubules. Score 3 indicated approximately half of the surface covered with SL and the other half with open tubules. Score 4 indicated SL covering a small portion of the surface with visible tubules, and Score 5 signified the absence of SL on the surface[9].

A single score for the amount of SL was assigned to each photomicrograph of each third. In instances of disagreement in scoring, the three examiners were required to reach a consensus. A total of 150 images (50 samples x 3 portions: cervical, middle, and apical) were analyzed twice at a 7-day interval.

Statistical analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). The data was analyzed using Kruskal-Wallis and Mann-Whitney U-tests. Statistical significance was established at $p<0.05$. Interexaminer agreement was evaluated by the Cohen-Kappa test.

Results

The kappa value for the interexaminer agreement was determined to be 0.89. Table 2 displays the means and standard deviations observed across the various groups and thirds for the different protocols employed. The highest values for SL removal were observed in the 17% EDTA + PUI group, followed by the 0.2% chitosan + PUI group and 0.2% chitosan group + CI. Overall, the cervical third exhibited more open tubules (2.91 ± 0.99), but there was no significant difference between the middle (2.51 ± 1.01) and apical (2.18 ± 1.11) thirds (P = 0.101). Photomicrographs obtained from the SEM evaluation, along with the corresponding scores, are depicted in Figure 1.

TABLE 2 · Means and standard deviation (SD) of smear score and score distribution according to root thirds and groups.

Groups	Coronal-third scores (%)					Middle-third scores (%)						Apical-third scores (%)						Total	
	1	2	3	4	5	Mean ± SD	1	2	3	4	5	Mean ± SD	1	2	3	4	5	Mean ± SD	Mean ± SD
Control	8.3	11.3	3.6	0.0	0.0	1.89 ± 0.60^{Ba}	12.5	11.3	0.0	0.0	0.0	1.67 ± 0.50^{Ba}	25	5.7	0.0	0.0	0.0	1.33 ± 0.50^{Ba}	1.63 ± 0.56^{B}
17% EDTA + CI	4.2	7.5	10.7	4.5	0.0	2.44 ± 0.88^{BCa}	12.5	11.3	0.0	0.0	0.0	1.67 ± 0.50^{Ba}	25	5.7	0.0	0.0	0.0	1.33 ± 0.50^{Ba}	1.81 ± 0.78^{BC}
0.2% chitosan + CI	0.0	5.7	17.9	4.5	0.0	2.78 ± 0.66^{BCa}	0.0	9.4	14.3	0.0	0.0	2.44 ± 0.52^{BCab}	12.5	9.4	9.4	3.6	0.0	1.78 ± 0.66^{BCb}	2.33 ± 0.73^{BC}
17% EDTA + PUI	0.0	0.0	3.6	13.6	62.5	4.44 ± 0.72^{Aa}	0.0	0.0	3.6	27.3	25.0	4.11 ± 0.60^{Aa}	0.0	0.0	0.0	3.6	12.5	4.00 ± 0.50^{Aa}	4.19 ± 0.62^{A}
0.2% chitosan + PUI	0.0	3.8	17.5	9.1	0.0	3.00 ± 0.70^{Ca}	0.0	7.5	14.3	4.5	0.0	2.67 ± 0.70^{Ca}	0.0	11.3	11.3	7.1	0.0	2.44 ± 0.72^{Ca}	2.70 ± 0.72^{C}

Capital letters indicate comparisons in the columns. Lower letters indicate comparisons in columns. Different letters indicate statistical differences ($p<0.05$). CI: conventional irrigation; PUI: passive ultrasonic irrigation.

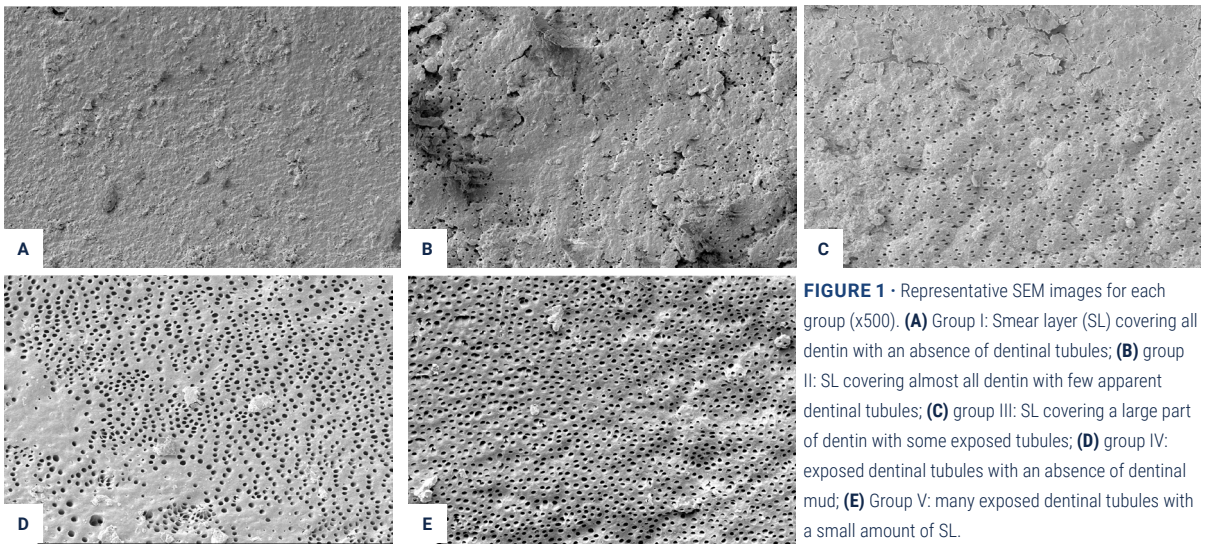

FIGURE 1 · Representative SEM images for each group (x500). **(A)** Group I: Smear layer (SL) covering all dentin with an absence of dentinal tubules; **(B)** group II: SL covering almost all dentin with few apparent dentinal tubules; **(C)** group III: SL covering a large part of dentin with some exposed tubules; **(D)** group IV: exposed dentinal tubules with an absence of dentinal mud; **(E)** Group V: many exposed dentinal tubules with a small amount of SL.

Discussion

One of the pivotal aspects during root canal treatment is the thorough removal of the SL, particularly in cases of infected canals[1,3]. This study aimed to assess the efficacy of 0.2% Chitosan and 17% EDTA solutions, with or without ultrasonic agitation, in SL removal. 17% EDTA + ultrasound protocol demonstrated the highest SL removal among all groups. Overall, utilizing ultrasound for solution agitation yielded the most favorable outcomes in SL removal. Additionally, SL removal was notably higher in the cervical third, followed by the middle and apical thirds. Thus, the null hypothesis was rejected.

The effectiveness of chelating agents is influenced by various factors, including application time, pH, concentration, and volume of the solution[8]. In this study, these parameters were determined based on previous research findings[8, 9]. The chelating solution was allowed to remain within the root canals for 3 minutes, as prolonged exposure beyond this duration, particularly with solutions like EDTA, does not enhance SL removal and leads to dentinal erosion[8,9].

EDTA stands out as the most extensively researched and utilized chelating solution for the removal of inorganic components from the SL[18]. It initiates a reaction with the calcium ions

present in dentin, forming calcium chelate, which effectively decalcifies the dentin to a depth of approximately 20 to 30 µm within a time frame of 3 to 5 minutes[19]. However, the use of EDTA has been scrutinized due to its erosive impact on dentin, its potential aggressiveness to periapical tissues, and its designation as a pollutant[18].

In this study, the Chitosan solution was prepared with a concentration of 0.2%. Notably, the solution was formulated using 1% acetic acid, stabilizing its pH at 3.2. Nevertheless, it's imperative to note that this pH stabilization alone cannot be solely attributed to SL removal efficacy, as comparative analysis against a 15% EDTA solution demonstrated superior SL removal results[9]. The chelating action of chitosan occurs through the binding of two amino groups of the Chitosan chain to the metal ion, facilitated by an anchoring system of the metal ion to the amino group. This ion exchange process is enabled by the presence of nitrogen atoms in the chitosan polymer, which possess free electron pairs[8].

A 2.5% NaOCl was employed following the chelating solution due to its remarkable capability to remove organic material. NaOCl exhibits unique properties that facilitate the transformation of necrotic debris and other protein components into soluble solutions, akin to substances like soap and chlorine. This transformation process effectively suspends these structures, enabling their subsequent aspiration[5,9,13].

Considering solely the solution used, the group utilizing 0.2% chitosan (group III) demonstrated higher SL removal compared to the group employing EDTA (group II). Vallabhaneni *et al.*[20] (2017) evaluated the effectiveness of 0.2% Chitosan, Smear Clear®, 0.1% citric acid, and 5.25% NaOCl in SL removal. Their findings indicated that Chitosan was only more effective than the NaOCl solution, suggesting that the residence time of the chitosan solution in the root canals may have been insufficient for complete SL removal. Similarly, Zhou *et al.*[10] (2018) compared

the effectiveness of the 0.2% chitosan and MTDA in SL removal of the root canals, concluding that chitosan was more effective than MTDA, particularly in the apical third. Additionally, Ozlek *et al.*[11] (2020) concluded in an in vitro study that final irrigation with 0.2% chitosan solution enhanced the displacement resistance of MTA - resin hybrid root canal sealer when compared to EDTA and citric acid.

There is limited literature directly confronting Chitosan with ultrasonic agitation in SL removal. However, in the present study, ultrasonic agitation notably improved SL removal when compared to groups without agitation. Pedro *et al.*[14] (2017) found that 0.2% Chitosan exhibited superior performance when ultrasonic agitation was employed. Moreover, Urban *et al.*[15] (2017) demonstrated that various agitation methods, including ultrasonic activation, significantly outperformed manual irrigation in SL removal. Abraham *et al.*[21] (2019), in contrast, reported that laser diode and EndoActivator were superior to ultrasonic agitation in SL removal when using 0.2% chitosan. While some literature reports similar efficiency between Chitosan and EDTA in terms of their behavior on the inorganic part and SL removal, discrepancies exist among studies[8,9,14].

SEM was selected as the evaluation method due to its widespread utilization in the analysis of SL removal[9,13,20,22]. A magnification of 500x was employed to minimize observational biases that could arise from larger magnifications, which may restrict the visualized area[12].

Under SEM observation, the group utilizing 17% EDTA with ultrasonic agitation exhibited minimal SL in all thirds of the root canals, with the cervical third displaying the most favorable outcomes. Ultrasonic agitation of the irrigation solution induces hydrodynamic turbulence, resulting in increased temperature and hydrostatic pressure, generating waves that dislodge debris from the dentin walls[16]. Conversely, significant amounts of SL were observed in all thirds of the root canals in the group

using 0.2% chitosan. These findings align with the study by Vallabhaneni *et al.*[20] (2017), which concluded that 0.2% Chitosan was less effective than 6% Citric Acid solution and Smear Clear® in SL removal across all three-thirds of the root canal. However, there are reports of the efficiency of chitosan in SL removal in the middle and apical thirds of the root canals, attributed to its demineralization capacity[9].

The cervical third consistently demonstrated greater SL removal across all groups. In a vitro study comparing the effectiveness of final irrigation with chitosan, EDTA, and citric acid on AH plus sealer penetration into dentinal tubules, Kesim *et al.*[23] (2018) concluded that all three substances improved the percentage of sealer penetration into the tubules in the coronal thirds[23]. This region offers better contact of the chelation solution with the dentin, despite the risk of solution loss, particularly during ultrasonic agitation. Conversely, the apical third showed minimal or no SL removal, consistent with previous findings[15]. Reduced diameter in this region influences the volume and contact of irrigating solutions with dentin walls. Additionally, the presence of bubbles formed during irrigation may hinder solution arrival in this region and contact dentin walls[13].

Considering the inherent moisture in vivo dentin, which can affect chelating agent penetration, simulating the oral environment is essential to develop clinical protocols closer to reality.

Conclusions

The combination of 17% EDTA + ultrasound resulted in significantly higher SL removal.

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Estudo comparativo da eficácia de protocolos de irrigação final na remoção da smear layer: um estudo por MEV

Resumo

Objetivo: Este estudo teve como objetivo avaliar a eficácia da quitosana 0,2% e do EDTA 17%, com ou sem ativação ultrassônica, na remoção da *smear layer* (SL). Métodos: Cinquenta incisivos bovinos foram seccionados a 17 mm de seus ápices, instrumentados utilizando instrumentos ProTaper Universal e divididos em cinco grupos (n=10): I- Água destilada + irrigação convencional (IC) (controle), II- EDTA 17% + IC, III- Quitosana 0,2% + IC, IV- EDTA 17% + irrigação ultrassônica passiva (IUP) e V- Quitosana 0,2% + IUP. As raízes foram divididas longitudinalmente e examinadas em um microscópio eletrônico de varredura com aumento de 500x para avaliar a remoção da SL. A presença de SL foi avaliada por três examinadores cegos usando uma escala de cinco pontos. Os dados foram analisados usando os testes de Kruskal-Wallis e Mann-Whitney U ($p<0,05$). Resultados: A análise estatística revelou diferenças significativas na remoção da SL entre os grupos ($p<0,05$). O grupo IV (EDTA 17% + IUP) apresentou eficácia de remoção significativamente maior do que os outros grupos ($p<0,05$). A remoção da SL foi maior no terço cervical em comparação aos terços médio e apical. Conclusões: O EDTA 17% combinado com IUP demonstrou superior remoção da SL.

PALAVRAS-CHAVE: Agentes Quelantes; Irrigação Ultrassônica Passiva; Smear Layer.

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