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Effect of glycolic acid as single irrigant versus sodium hypochlorite and EDTA on enterococcus faecalis count and smear layer removal in single-rooted teeth (an *in vitro* study)

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Abstract

Aim: The study aimed to compare 10% Glycolic acid as a single irrigant versus 2.5% sodium hypochlorite followed by 17% Ethylenediaminetetraacetic acid (EDTA) in terms of *E. faecalis* count reduction and smear layer removal in single-rooted teeth.

Methodology: Thirty human extracted mandibular premolars were prepared and inoculated with *E. faecalis* for one week to evaluate *E. faecalis* count reduction. Teeth were randomly assigned to two groups (n = 15) according to the irrigation protocol used. Group I: intervention (10% Glycolic acid) and Group II: Control group (Irrigation using 2.5% NaOCl followed by 17% EDTA). After 24 hours of incubation, bacterial count reduction using colony forming units CFUs/ml was determined. For evaluating the ability of irrigants to remove the smear layer, another twenty-two mandibular premolars were chosen and randomized into two groups (n = 11). During “chemo-mechanical” preparations, the root canals were irrigated with different irrigation protocols according to each group. Then, the roots were split longitudinally in the bucco-lingual direction into two halves. A smear layer assessment was conducted on the “coronal, middle, and apical thirds” of the root canal using an Environmental Scanning-Electron Microscope (ESEM) at 1000x magnification.

Results: Regarding *E. Faecalis* count reduction, there was “no significant” difference between the two groups ($p = 0.14$). Regarding the ability to remove the “smear layer”, there was no significant difference between the two groups at coronal, middle and apical. While comparing smear layer scores within the same group, the “apical third” showed a significantly higher smear layer score in the two groups.

Conclusion: Glycolic acid can be used as a single irrigant owing to its antibacterial efficacy and the ability to remove the smear layer.

Keywords: Glycolic acid, NaOCl, EDTA, *E. faecalis*, smear layer, endodontic irrigation

Introduction

Endodontic treatment aims to eradicate all “microorganisms” from the entire root canal system. This is achieved by cleaning and shaping as well as three-dimensional obturation of the root canal space [1].

One of the major obstacles in endodontic treatment is the presence of bacteria, particularly “*Enterococcus faecalis* (*E. faecalis*)” [2]. They are gram-positive facultative anaerobic bacteria that can penetrate the dentinal tubules and form biofilm [3]. They exhibit various resistance mechanisms, such as using fluid from dentinal tubules and the periodontal ligament to develop biofilms, which helps them hide from the host defense mechanism and antimicrobial agents [4]. This is further complicated by the identification of a strain of *E. Faecalis* capable of producing calcified biofilm through increased precipitation of hydroxyapatite in biofilm, making it resistant to healing [5]. These bacteria can survive for prolonged periods of time without adequate nutrition until an adequate supply is provided [6]. As a result, the bacteria are able to escape the action of instrumentation, irrigation, and medications [7, 8].

Irrigation plays a crucial role in the successful root canal treatment as it has several important mechanical, chemical and biological functions. Also, it is the only method of reaching the areas of the root canal wall that remain untouched by mechanical instrumentation. An ideal irrigant has “antimicrobial properties” and the ability to remove the “smear layer” [9].

The most common irrigation protocol used in endodontics is the combination of “sodium hypochlorite (NaOCl)” and ethylenediaminetetraacetic acid (EDTA). “Sodium hypochlorite” is considered the gold standard endodontic irrigation solution owing to its organic solvent action, potent “antimicrobial activity” even at low concentrations, proteolytic properties, ability to disintegrate pulpal tissues, and good lubricating ability. Moreover, it removes exposed collagen fibrils, reducing the amount of collagen available for *E. Faecalis* adhesion [10]. However, its unpleasant smell and taste, relative toxicity, potential allergic reactions, and ability to cause burning of surrounding tissues have been criticized. Additionally, it has no impact on the inorganic components of the “smear layer”. Therefore, numerous studies are currently being conducted to identify an effective organic alternative to sodium hypochlorite [11].

The smear layer is an amorphous and irregular layer that forms on the root canal walls after mechanical preparation. It is composed of dentin, necrotic tissue, remnants of “odontoblastic processes”, “pulp tissue”, and microorganisms. The structure of the “smear layer” consists of two distinct parts: a superficial part that is poorly adherent and another part in the form of plugs in the dentinal tubules for a few micro-meters [12]. The thickness of the “smear layer” ranges from 1-5 μm [13]. The presence of the “smear layer” restricts the penetration of irrigants and medicaments into the dentinal tubules. Therefore, removing the “smear layer” is recommended and can be achieved by using an EDTA irrigant [14].

Ethylenediaminetetraacetic acid (EDTA) is a commonly used final irrigant solution in endodontics. It is considered the gold standard irrigant for removing the “smear layer” because of its powerful chelating ability. EDTA reacts with calcium ions to remove the inorganic component of the “smear layer”. However, EDTA cannot remove the organic component of the “smear layer”, so it should be used in conjunction with a proteolytic material like NaOCl [15]. EDTA is widely used as a lubricant during rotary Nickel Titanium (NiTi) instrumentation and in the negotiation of calcified root canals, in addition to its application for smear layer removal [16]. However, it may cause alterations in the dentinal wall's micro-hardness and mineral content [17]. EDTA is produced through an industrial synthesis process that involves ethylenediamine, formaldehyde, and sodium cyanide. This process results in residual compounds that may cause harm to the environment in which the EDTA is discharged [18].

Several studies have been conducted over the years to identify possible novel irrigants that might substitute conventional endodontic irrigants. The available literature indicates the benefits and drawbacks of each irrigant. Today, using a single irrigant rather than dual irrigants is a valid choice. This approach helps to avoid the drawbacks of combined irrigants and reduces chair-side time, resulting in a more convenient healthcare experience for patients. One such irrigant is Peracetic acid (PAA), which has been found to remove the “smear layer” [19] and exhibit excellent antimicrobial effects [20].

Glycolic acid (GA) is an organic substance extracted from sugar cane and certain other vegetables [21]. It has wide-ranging applications in dermatology, from skin moisturizing to deep chemical peeling [22], owing to its “anti-inflammatory, keratolytic, and antioxidant” properties. GA works by targeting the stratum corneum, improving breakdown and reducing cohesiveness, and eventually causing desquamation [23]. In dentistry, glycolic acid is used in the restorative

procedures to etch enamel and dentin [24]. In addition, it is used in the endodontic treatment procedures to remove the “smear layer” formed on the dentine canal walls [25]. Furthermore, GA is readily biodegradable, making it environment friendly unlike EDTA that exhibits waste disposal problems [26]. Moreover, glycolic acid can eliminate “gram-positive” and “gram-negative bacteria” [27]. The activity of this acid is based on eliminating free radicals [28]. Additionally, glycolic acid is a potential alternative to dual irrigant solutions, being used alone thus reducing the chair side time. Above all, it will overcome the possible drawbacks of the other two irrigants used.

Currently there is no research conducted on the use of glycolic acid as a single irrigant. Hence, the purpose of this “*in vitro* study” was to compare the ability of 10% glycolic acid as a single irrigant to 2.5% NaOCl followed by 17% EDTA in terms of antimicrobial efficacy and removal of “smear layer”.

Materials and Methods

The study received approval from “the research ethics committee at Cairo University's Faculty of Dentistry”, with the assigned approval number of 25-9-21.

Sample selection

Freshly extracted human mandibular permanent, single-rooted premolar teeth with mature apices were collected from the dental clinic of the “National Diabetes and Endocrinology Institute in Cairo” and the “Oral Surgery Department of the Faculty of Dentistry at Cairo University”. These teeth were extracted due to orthodontic or periodontal problems. Teeth with caries or previous restorations, fractures, enamel crazing, root resorption, obliterated canals, or previous “root canal treatment” were excluded. The teeth were cleaned of any hard debris using an “ultrasonic scaler” (Woodpecker, China), and then immersed in “5.25% NaOCl” for 30 minutes to facilitate removal of soft periodontal tissues and debris. The teeth were kept in a saline solution until they were ready to be used to maintain their natural hydration.

Sample preparation for Bacterial count (*E. faecalis*) reduction

Thirty human mandibular premolars with a single root and canal were selected after radiographic examination. The teeth were decoronated using a low-speed diamond saw (CUTFLEX® diamond discs, Dental Future Systems DFS DIAMON, Germany) under copious irrigation to obtain uniform root lengths of 16 mm, as measured by an endometer. A K-file size 10 (K-file size 10, Mani® K-FILES, MANI Inc., Japan) was inserted into the root canal to check patency, then the “working length” (WL) was adjusted using a K-file #15 (K-file size 15, Mani® K-FILES, MANI Inc., Japan) until showing from the apex and subtracting 1mm, so a standardized WL of 15 mm was obtained. The root canals were instrumented using the “ProTaper Next rotary system (Dentsply Maillifer, Ballaigues, Switzerland)”. The procedure began with an X1 (17/.04) file and ended with an X4 (40/.06) file, with a speed of 300 rpm and 2-2.5 N.cm torque, following the manufacturer's instructions with the XSMART Endo Motor (Dentsply Maillifer, Ballaigues, Switzerland). Copious irrigation with 3 mL of freshly prepared 2.5% sodium hypochlorite solution was done between each instrument using a “30-gauge-max-i-probe side vented needle (Dentsply Maillifer, Ballaigues, Switzerland)”. The needle was inserted 1mm short from the WL. “Apical patency” was

retained by using a “#10 K-file” between each rotary file. After root canal instrumentation was completed, the teeth were irrigated with 5mL of normal saline (0.9% NaCl). Then, the “smear layer” was removed using 5ml “17% (EDTA)” solution (Prevest Direct, India) for 1 min. Subsequently, 5 mL of saline was used to irrigate the root canals again to inactivate the effect of EDTA, and the canals were dried with x4 paper points (Dentsply Sirona, Ballaigues, Switzerland). Sealing of the apical foramen was performed by applying a flowable composite (3M Dental Products, St. Paul, MN) on the root apex to avoid overflow of the test substances during the experiment. Two layers of cyanoacrylate-based adhesive (3M Dental Products, St. Paul, MN) were applied to the external surface of the roots except for the cervical region surrounding the root canal orifice.

Sample Sterilization

The roots were sterilized at 120°C in an autoclave for 30 minutes.

Culture and inoculum preparation

A suspension was prepared by adding 1 ml of a pure culture of *E. Faecalis* (ATCC 29212), which was grown in sterile “brain-heart infusion broth” (BHI) for 24 hours to match the turbidity of 1.5×10^8 CFU/mL (equivalent to ± 0.5 McFarland standard). The purity of the culture was confirmed by examining colonies of *E. Faecalis* by three tests: Bile esculin test, the cysteine lactose electrolyte-deficient agar (CLED test) and A gram-stained film test. Once the purity of the culture was confirmed all “root canals” were filled with 30 μ “*E. faecalis*” suspension by using a micropipette. The infected teeth were inoculated with this solution, then placed inside a sterile “Eppendorf” tube and kept in an incubator (Fisher ISOTEP, Incubator. WTC Binder, Tuttlingen/Germany) aerobically for one week at 37 °C with refreshment by sterile BHI media every 48 hours.

Experimental groups

Under aseptic conditions, using new sterile gloves and sterile tweezers to hold each cultured tooth to avoid any contamination of the specimens, two specimens were collected from the canals using the “paper point method” S1: before irrigation. S2: after irrigation.

Sample (S1) was obtained by gently rinsing the root canals with 1 mL of saline to remove any non-adherent cells. After that, three paper points #25 (Dentsply Sirona, Ballaigues, Switzerland) were sequentially used to collect the initial sample. The three points were placed subsequently to WL and maintained in the canal for 30 sec each. The paper points collected from each tooth were placed into individual Eppendorf tubes containing “phosphate-buffered saline” (PBS) (HiMedi® Laboratories Pvt. Ltd. Mumbai, India). The roots were divided randomly into 2 groups (n = 15) and each irrigant was assigned to one group. Group I: 10 ml of 10% Glycolic acid (GA) Group II: Control group (C) total volume of 10 ml (5 ml of 2.5% NaOCl followed by final irrigation with 5 ml of 17% EDTA). To separate between them, 5 ml of saline solution was used in between. The canals were filled with the assigned irrigant, which was renewed every one minute for a total application time of 3 minutes. All irrigants were applied into the canal with a 30-gauge-max-i-probe side vented needle in a plastic syringe. Lastly, saline was used as a final flush to remove any chemicals from the canals prior to S2 collection. The postoperative samples (S2) were taken after irrigation, using the same procedure

mentioned before.

Sample preparation for smear layer removal:

Another twenty-two human teeth with a single root were collected and prepared as previously mentioned. The mechanical procedure was the same as previously mentioned in Sample preparation for bacterial count, except that the “root canals” were irrigated with irrigant solution at each change of rotary files according to the group the specimen was assigned to. During the experiment, in Group II: The “root canals” were irrigated each time the rotary files were changed using a total volume of “5ml of 2.5% NaOCl followed by 5 ml of 17% EDTA” as the final irrigating solution, with saline solution used in between. After root chemo-mechanical preparation was completed, the teeth were irrigated with 5 mL of saline to remove any residue and dried with x4 paper points in both groups.

Specimen preparation for ESEM assessment of smear layer removal

After different irrigation protocols, the paper points were left inside the root canals to prevent the dentin dust penetrating into the areas of root evaluation during splitting. Then roots were split longitudinally by applying buccal and lingual grooves on the external root surface using a double-faced diamond disc at low speed under profuse irrigation. This was done without entering the canal lumen. After that, a hammer and a microtome blade were used to complete the division of the root into two halves Figure (1). The two halves of each root were then examined under stereo microscope (Lecia microsystems, Switzerland) under magnification of (X16) to select the most representative half to be used for ESEM analysis. The specimens were dehydrated, fixed on metal stubs with electro-conductor glue and then transferred to ESEM plate. Environmental Scanning electron microscopy (ESEM) “Quanta 3D 200i (FEI company, Hillsboro, Oregon, USA)” was used with an acceleration voltage of 20 K.V. All the specimens were scanned at a magnification of 1,000 x. “The coronal, middle and apical thirds” of the root canal were examined individually at 3, 7, and 12 mm from the apex respectively, in each specimen at magnifications of 1,000 x^[29]. Finally, ESEM images were obtained and analyzed.

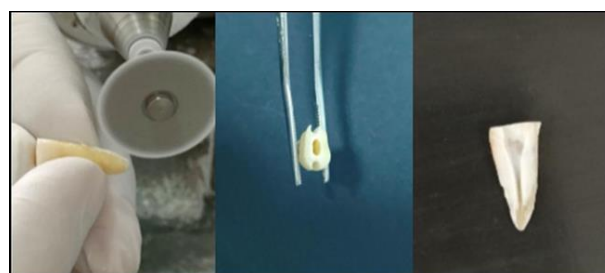


Fig 1: Sectioning each root longitudinally



Fig 2: Specimens glued on metal stubs using electro-conductor glue and then transferred to ESEM plate

Evaluation of bacterial count reduction

Bacterial count reduction evaluation using the brain heart infusion agar method. In the laboratory, all groups were sampled to count the number of bacteria remaining in the canals. To collect the samples, a sterile 0.9% saline solution was injected into each canal and three sterile paper points were inserted into WL. After that, paper points were put into sterile eppendorf tube containing a 500 µL solution of (PBS), and they were vortexed for one minute. To assess the microbiological load, 100 µl aliquots of the vortexed samples were inserted in a fresh sterile eppendorf tube with 1 ml of thioglycolate to produce a 1/10 concentration. Sterile micropipettes with yellow tips were used to collect 20 µl from eppendorf tube. The sample was then distributed over the surface of the brain-heart infusion agar plates (TM MEDIA®, TITAN BIOTECH LTD. Rajasthan, India) by using a sterile L-shaped glass rod. The plates were then incubated at 37°C for 24 hours. Each plate's bacterial colonies were counted and reported as CFU/ml^[30].

Evaluation of smear layer removal

The ESEM photographs were evaluated by two blinded observers using a scoring method to assess the presence of “smear layer”. Each sample was assigned a “smear layer” score based on the 3-level scoring system established by Zmener *et al.*, 2005^[31] was used as follows: Score 1: “All dentinal tubules were open, and no smear layer was present”. Score 2: “Some dentinal tubules were open, and the rest was covered by a smear layer”. Score 3: “Continuous smear layer covered the canal walls, and no dentinal tubules were seen”. Each examiner rated each micrograph independently and blindly. Three micrographs served as visual reference standards for the scoring system. When there was a discrepancy amongst the examiners, the micrograph in question was addressed until an agreement on a specific score was obtained. Finally, the micrographs were decoded and the results tallied.

Results

Bacterial count reduction

The findings from the bacterial count reduction analysis demonstrated that there was “no statistically significant difference” between the two groups ($p = 0.949$) before the application of irrigation protocol Table (1). Regarding the bacterial count post-irrigation, there was “no statistically significant difference” between glycolic acid and NaOCl followed by EDTA ($p = 0.14$) Table (2). While none of the experimental irrigation protocols achieved 100% eradication of “*E. faecalis*”, however, there was significant reduction in bacterial count from pre-operative to post-irrigation within each group ($p < 0.001$) Table (3).

Table 1: Descriptive statistics, 95% confidence interval and the result of “independent t-test” for comparison of preoperative bacterial count between the two groups:

	Group GA	Group C	P - Value
Mean (SD)	813.5 (40.8)	812.5 (38.6)	
Median (Range)	814 (762 - 876)	811 (767 - 893)	0.949
95% CI	(790.9 - 836.1)	(791.1 - 833.9)	

“*P: probability level which is significant at $P \leq 0.05$; **Bacterial counts in 10^4 CFU/ml.”

GA, Glycolic acid; C, Control group

Table 2: Descriptive statistics, 95% confidence interval and the result of independent t test for comparison of post-irrigation bacterial count between the two groups:

	Group GA	Group C	P - Value
Mean (SD)	82.5 (13.7)	76.1 (9)	
Median (Range)	85 (64 - 110)	77 (62 - 88)	0.14
95% CI	(74.9 - 90.1)	(71.1 - 81)	

“*P: probability level which is significant at $P \leq 0.05$; **Bacterial counts in 10^4 CFU/ml.”

GA, Glycolic acid; C, Control group

Table 3: The mean, standard deviation, and results of paired t test for comparison of bacterial counts within each group

	Preoperative bacterial count	Post-irrigation bacterial count	P - Value
	Mean (SD)	Mean (SD)	
Group GA	813.5 (40.8)	82.5 (13.7)	<0.001*
Group C	812.5 (38.6)	76.1 (9)	<0.001*

“*P: probability level which is significant at $P \leq 0.05$; **Bacterial counts in 10^4 CFU/ml.”

GA, Glycolic acid; C, Control group.

Regarding the “smear layer removal”, there was “no significant difference” between the two groups in all thirds, as demonstrated in Figure 1) and Table (4). While the “smear layer” scores within the same group showed in the glycolic acid group, the “apical third” showed a significantly higher score than the “coronal and middle thirds”. However, there was “no significant difference” between the “coronal and middle thirds”. In the control group, there was “no significant difference” in “smear layer” score between the apical and middle thirds, but the score in “the coronal third” was significantly lower than that in the “middle and apical thirds” Table (5).

Table 4: Descriptive statistics, 95% confidence interval and the result of “Mann - Whitney U test” for comparison of “smear layer” scores between the two groups

	Group GA	Group C	P - Value
Coronal third			
Mean (SD)	1 (0)	1 (0)	
Median (Range)	1 (1 - 1)	1 (1 - 1)	1.0
95% CI	(1 - 1)	(1 - 1)	
Middle third			
Mean (SD)	1.5 (0.5)	1.8 (0.6)	
Median (Range)	1 (1 - 2)	2 (1 - 3)	0.155
95% CI	(1.1 - 1.8)	(1.4 - 2.2)	
Apical third			
Mean (SD)	2.3 (0.5)	2.5 (0.5)	
Median (Range)	2 (2 - 3)	3 (2 - 3)	0.204
95% CI	(2 - 2.6)	(2.2 - 2.9)	

GA, Glycolic acid; C, Control group. “P: probability level which is significant at $P \leq 0.05$ ”

Table 5: Means, standard deviations and the results of “Friedman’s test” and “post hoc Wilcoxon’s signed rank tests” with Bonferroni correction for comparison of “smear layer” scores within each group:

	Coronal	Middle	Apical	P - Value
Group GA	1 ^b (0)	1.5 ^b (0.5)	2.3 ^a (0.5)	<0.001*
Group C	1 ^b (0)	1.8 ^a (0.6)	2.5 ^a (0.5)	<0.001*

*Significant at $p < 0.05$; **Different small letters indicate statistical significance within rows”

GA, Glycolic acid; C, Control group

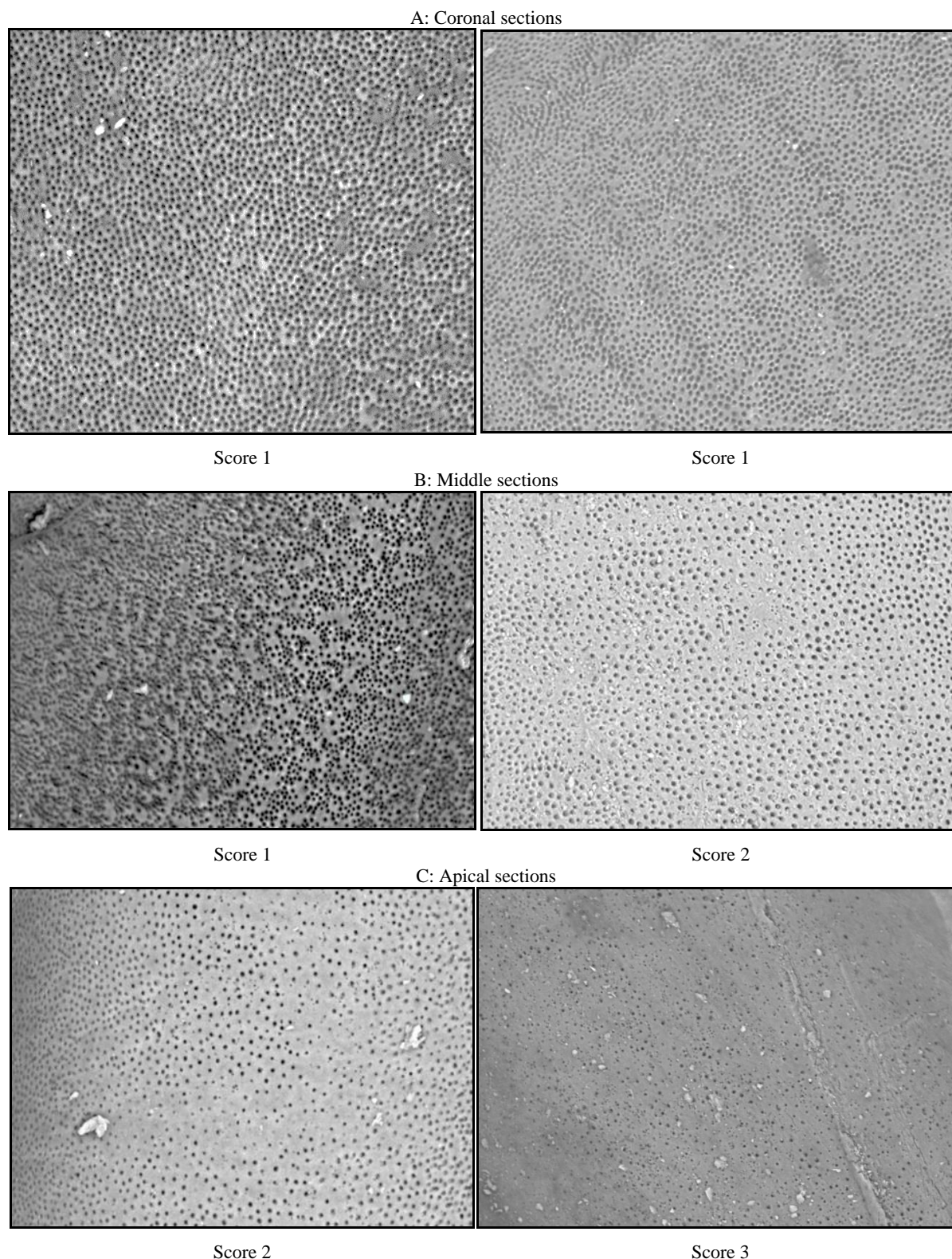


Fig 3: ESEM micrographs (1,000 x) showing “smear layer removal” in samples of glycolic group on left side and the control group on right side (A) Coronal third, (B) Middle third, and (C) Apical third

Discussion

This study includes single-rooted mandibular premolars because they have a single oval canal cross-section, which cannot be touched and cleaned well by the rounded cross-sectioned endodontic file. As a result, there are substantial areas of the canal walls that remain untouched, leading to an accumulation of hard-tissue debris in irregularities within the root canal space [32, 33]. According to literature, these untouched zones may reach up to 35% of the overall canal walls [32]. Hence, the effectiveness and function of the

irrigants would be more reliable.

For the DE coronation procedure, teeth with lengths between 18mm and 25mm were chosen so that the resulting roots would measure 16 ± 1 mm. This is because the average length of mandibular premolars is 22.5mm according to Cleghorn *et al.*, 2019 [34].

ProTaper Next rotary system was employed for mechanical preparation to remove pulp tissue, standardize the canal diameter and provide a more predictable preparation form while also increasing procedural efficiency [35, 36]. This is

because of the offset mass rotation motion, which produces a unique asymmetrical rotary motion and at any given cross-section the file only touches the wall at two points; which gives the advantage of reducing the taper lock and the file has more cross-sectional space for better cutting and loading debris [37].

Apical preparation was completed with an X4 file as a master apical file, which is equivalent to size #40 ISO, to achieve proper preparation because the surface area of the canal left untouched was 35%–50% with large apical preparations (minimum ISO #40) and preparations less than file #40, which leave much more unprepared dentin [38]. According to the findings of the research conducted by Usman *et al.*, 2004 [39] “root canals” prepared to “size #20” exhibited considerably higher apical debris than those produced to size #40, even when the taper was set at 0.06. Therefore, the common rule of making the canal instruments three sizes larger than the initial file may not clean the dentinal walls adequately. Additionally, wider apical preparations minimize the number of bacteria because leaving bacteria in the apical part of the root may contribute to failure. Larger apical preparations are essential to remove contaminated dentin and enable deeper irrigant flow. Moreover, when root canals were prepared less than size #40, syringe irrigation was shown to be less effective [38].

A flowable composite was used to seal the apex to simulate a clinical situation in which the root apex is encircled by periodontal ligaments and enclosed in the alveolar bone socket [40]. Meanwhile, the flowable composite maintained the inoculated bacteria enclosed within the root canal system [41].

Sample Sterilization was done after the chemo-mechanical preparation using an autoclave to ensure a completely sterile root canal system prior to bacterial inoculation [42].

This study investigated *E. faecalis* since it has various characteristics that contribute to its virulence, including the capacity to survive in conditions with limited nutrition supplies as well as the strong ability to penetrate the dentinal tubules and form biofilm causing endodontic treatment failure [36, 43, 44].

A Colony-Forming Unit (CFU) was used to evaluate bacterial count reduction, which is regarded as a standard approach for evaluating antibacterial efficacy since it effectively quantifies the quantity of bacterial content and provides an indication of the low viable bacteria load of each evaluated irrigation protocol. However, samples were obtained from the main root canal only, therefore it is impossible to evaluate how well the irrigation procedure affects the depth of the dentinal tubules [36].

The sample was inoculated with *E. faecalis* for one week to allow maximum growth of the *E. Faecalis* [45].

The BHI agar method was utilized in this study since it is one of the most used techniques for determining the antimicrobial activity of endodontic irrigant. It enables direct comparisons of the test irrigants against the test microorganisms, indicating which substance can eradicate bacteria in the environment of the “root canal system” [46].

In this study, 5 mL of 2.5% NaOCl was used between every two successful files in the control group as it is the gold standard endodontic irrigant owing to its antibacterial efficacy and its capacity to disintegrate necrotic pulp tissue and neutralize bacterial byproducts. According to a study conducted by Vaziri *et al.*, 2012 [47], 2.5% NaOCl has a strong ability to disintegrate organic tissues and denature bacterial toxins. Moreover, NaOCl has a powerful effect even in non-instrumented areas. On the other hand, NaOCl cannot remove

the “smear layer” as evident in the current literature [48, 49].

17% EDTA is the most popular chelating agent used as a final irrigant, which has a powerful capacity to remove the “smear layer”. However, it has a limited capacity to promote bacterial reduction, is cytotoxic, causes extensive changes in the dentinal structure and is still a pollutant after it is made [49].

Glycolic acid as a single irrigant shows superior antibacterial efficacy as it has low pH, high solubility, and its derivatives are employed in the production of antibiotics [27, 36]. Furthermore, bacterial reproduction is largely dependent on the union of amino acids to make proteins. This chain may be disrupted at any point by GA, which limits the production of bacterial proteins and so neutralizes bacteria [36]. Moreover GA can “remove the smear layer” by dentinal decalcification and eliminating the inorganic portion of the “smear layer” [21].

The environmental SEM (ESEM) was used in this study, as it may provide subjective findings based on the selection of the investigated areas as well as the operator's interpretation of the data. To standardize the readings, the images were taken at fixed lengths from the root apex (3, 7 and 12 mm from the root apex) in this research. Furthermore, To maximize the reliability and precision of the findings, the SEM examination was conducted by two experienced and blinded examiners [50].

One of the most significant potential benefits of using the ESEM over Scanning electron microscopy (SEM) is the ability to scan samples without pretreatment like placing conducting coatings, which eliminates the possibility of artifacts produced during the process of SEM sample preparation [51]. Moreover, the environmental scanning electron microscope provides technology for imaging hydrated or dehydrated samples with minimal manipulation and time [52]. The use of low vacuum and low voltage results in the production of high-quality images that are less electrostatically distorted and have a spatial resolution as low as 1.5 mm, which is 3-6 times better than SEM [53].

Studies used magnifications ranging from low to high magnification power. Low magnification power examines a broad surface area but lacks surface detail and vice versa for the high magnification power. Magnification of 1,000 x was chosen in the present investigation to inspect the “smear layer” since it yields a large surface area with distinguished details [54, 55].

A scoring system is a standard approach for the evaluation of “smear layer removal” [56]. In this research, the score scale established by Zmener *et al.*, 2005 [31] was used owing to its clarity, accuracy and simplicity as evident in literature [57-59].

The bacterial count results showed that there was “no statistically significant difference” between the two groups before the application of irrigation protocol; where the CFU count was 813.5 and 812.5 for the control and intervention groups respectively. These results confirm the viability and proper cultures of bacteria with no bias between the two tested groups. The results coincide with the previous studies conducted by Gambin *et al.* 2020 [30] and Souza *et al.* 2021 [36] in which bacterial count was applied before irrigation to check the viability and purity of the microorganisms in the canals.

The findings of this study indicate that there was “no significant difference” in bacterial count between the use of glycolic acid and NaOCl followed by EDTA during post-irrigation. The mean CFUs were 76.1 and 82.5, respectively, indicating a significant reduction in *E. faecalis* count post-irrigation, which is consistent with previous studies conducted by Elkabbany 2022 [45] and Abdel Hafez *et al.*, 2019 [60]. However, our results disagreed with the study conducted by

Gambin *et al.*, 2020^[30], which showed a significantly higher reduction in bacterial count in the NaOCl group. This could be attributed to their use of NaOCl, GA, and EDTA each as a separate irrigant, as well as the use of 3ml of NaOCl at a concentration of 6% instead of the 5ml of NaOCl at a concentration of 2.5% used in this study. Furthermore, in the previous study by Gambin *et al.*, 2020^[30], there was a “significant difference” between EDTA and glycolic acid, which contradicts the findings of our study. This difference could be due to the use of EDTA only in irrigation of the canal without preceding NaOCl, whereas NaOCl was followed by EDTA as a routine irrigation protocol in our daily practice. In add Vition, Souza *et al.* 2021^[36] compared glycolic acid and EDTA and concluded that there was a significant increase in bacterial reduction for the glycolic acid group, which contradicts the findings of our study. This difference may be due to the use of EDTA as a single irrigant without preceding NaOCl, unlike in our study. As known, EDTA is a chelating agent with no antibacterial efficacy against *E. faecalis*^[61-63].

The results of the present study showed that none of the experimental irrigation protocols obtained 100% eradication of *E. faecalis* but there was significant reduction in bacterial count from preoperative to post-irrigation within each group. Whereas, for the glycolic acid group, the CFU before irrigation was 813.5 while post irrigation mean reduction was 82.5 with significant reduction ($p < 0.001$). Similarly, in the control group, the CFU before irrigation was 812.5 while post irrigation mean reduction was 76.1 with significant reduction ($p < 0.001$). The findings of the current study are accordant with these studies Souza *et al.* 2021^[36], Gambin *et al.* 2020^[30], Souza *et al.*, 2018^[61] Zhang *et al.*, 2015^[64], Cecchin *et al.*, 2015^[65] Haapasalo *et al.*, 2014^[63]. The antibacterial efficacy of the tested irrigants may be interpreted by that NaOCl causes a shift in the oxidation-reduction potential of the bacterial cell where sodium ions rapidly diffuse through the cell wall of the bacteria causing its death^[61, 66] and GA suppresses the protein synthesis of the bacteria cell^[67, 68].

Regarding the removal of the “smear layer”, there was “no significant difference” between the two groups, in the “coronal, middle and apical thirds”. These findings are consistent with earlier research by Barcellos *et al.*, 2020^[21] and Dal Bello *et al.*, 2019^[69]. This is interpreted as the ability of the GA to remove the inorganic component of the “smear layer” and decalcify dentin by removing calcium ions from it^[21], which is comparable according to the results obtained to the effect of EDTA by removing inorganic parts from the “smear layer” via reaction with calcium ions to form soluble calcium chelates.

The results of the glycolic acid irrigant revealed a significantly higher “smear layer” score in the “apical third” than in the “coronal” and “middle thirds”. This finding is in accordance with previous study conducted by Barcellos *et al.*, 2020^[21]. Incontrast, the study by Dal Bello *et al.*, 2019^[69] showed “no significant” difference between “the middle and apical thirds”, which is discordant with our study. This difference may be justified using different electron microscopes to capture the images. ESEM used in this study requires no pretreatment of the samples, unlike SEM which mandates preparation of the sample that, in turn, may negatively affect the surface details. Moreover, the magnification power used in the current study was 1,000 x while that in the previous study was 2,000 x. Additionally, different scoring systems were used to assess “smear layer removal”, such as Zmener *et al.*, 2005^[31] scoring system used

in this study and Hülsmann *et al.*, 1997^[70] scoring system used in the previous study. Our findings can be interpreted by the fact that the “smear layer” cannot be completely removed from the apical third, due to the presence of sclerotic dentin in the apical part of the canal impeding the irrigants’ flow^[49,71] and larger width of the canal at the “coronal and middle thirds” in comparison to the “apical third”, that is the narrowest area, allowing higher flow of the irrigants^[21, 72, 73]. Within the control group, there was “no significant difference” in “smear layer” score between “the apical and middle thirds”, while “the coronal third” showed significantly lower “smear layer” score “than the middle and apical thirds”, which is in accordance with Dal Bello *et al.*, 2019^[69]. While study conducted by Barcellos *et al.*, 2020^[21] showed all experimental irrigant less effective in removing the “smear layer” on the “apical third” of the root canal compared to “the coronal and middle thirds”.

The current study accepted the null hypothesis, which stated that there was “no significant difference” between using 10% glycolic acid versus 2.5% sodium hypochlorite and 17% EDTA in terms of reducing *E. faecalis* count and removing “smear layer”. However, this highlights the positive effect of glycolic acid which can be used as a single irrigant owing to its antibacterial effect and “smear layer removal” comparable to NaOCl and EDTA.

Conclusion

- Within the limitations of this in-vitro study, it can be concluded that, Glycolic acid can be used as a single irrigant owing to its antibacterial efficacy and the ability to remove the “smear layer”.
- Up to date, no irrigant achieves total eradication of *E. faecalis*.
- “Smear layer” is more prevalent in the apical third than the coronal third of the dentinal root canal.

Recommendations

Under the conditions of the current study, we recommend further research to explore the antibacterial effectiveness and removal of “smear layer” of glycolic acid in conjunction with different methods of irrigant activation, such as sonic activation, laser, and brush file. Additionally, further research is required using irrigants with different concentrations, pH, contact time, and higher temperatures. Furthermore, we recommend conducting further research comparing the efficacy of glycolic acid with other single irrigants, such as PAA. In addition to the previous, we recommend evaluating the effect of glycolic acid as a “single irrigant” on the quality of sealer penetration within dentinal tubules. Moreover, we suggest using the percentage of open dentinal tubules with digitized software as an assessment method for evaluating “smear layer” removal.

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Conflict of Interest

The authors state that they do not have any no conflict of interest regarding the publication of this paper

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References

1. Haapasalo M, Endal U, Zandi H, Coil JM. Eradication of

- endodontic infection by instrumentation and irrigation solutions. *Endod Topics*. 2005;10(1):77-102.
2. Portenier I, Waltimo TM, Haapasalo M. Enterococcus faecalis—the root canal survivor and ‘star’ in post-treatment disease. *Endod Topics*. 2003;6(1):135-59.
 3. Hubble T, Hatton J, Nallapareddy S, Murray B, Gillespie M. Influence of enterococcus faecalis proteases and the collagen-binding protein, ace, on adhesion to dentin. *Oral Microbiol Immunol*. 2003;18(2):121-6.
 4. Narayanan LL, Vaishnavi C. Endodontic microbiology. *J Conserv Dent*. 13:233-9.
 5. Del Fabbro M, Samaranayake LP, Lolato A, Weinstein T, Taschieri S. Analysis of the secondary endodontic lesions focusing on the extraradicular microorganisms: an overview. *J Investig Clin Dent*. 2014 Nov;5(4):245-54.
 6. Figdor D, Davies JK, Sundqvist G. Starvation survival, growth and recovery of enterococcus faecalis in human serum. *Oral Microbiol Immunol*. 2003;18(4):234-9.
 7. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. *J Endod*. 2002;28(10):689-93.
 8. Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in enterococcus. *Virulence*. 2012;3(5):421-569.
 9. Topbas C, Adiguzel O. Endodontic irrigation solutions: A review: Endodontic irrigation solutions. *Int J Dent Res*. 2017;7(3):54-61.
 10. Iqbal A. Antimicrobial irrigants in the endodontic therapy. *Int. j. health sci*. 2012 Jul 1;6(2):186-92.
 11. Fedorowicz Z, Nasser M, Sequeira-Byron P, de Souza RF, Carter B, Heft M. Irrigants for non-surgical root canal treatment in mature permanent teeth. *Cochrane Database Syst Rev*; c2012. p. 9.
 12. Cameron JA. The use of ultrasound for the removal of the smear layer. The effect of sodium hypochlorite concentration; SEM study. *Aust. Dent. J*. 1988 Jun;33(3):193-200.
 13. Mader CL, Baumgartner JC, Peters DD. Scanning electron microscopic investigation of the smeared layer on root canal walls. *J. Endod*. 1984 Oct 1;10(10):477-83.
 14. Alamoudi RA. The smear layer in endodontic: To keep or remove—an updated overview. *Saudi Endod J*. 2019;9(2):71-81.
 15. Doumani M, Habib A, Doumani A, Kinan M. A review: The applications of edta in endodontics (Part I). *IOSR-JDMS*. 2017;16(9):83-5.
 16. Peters OA, Boessler C, Zehnder M. Effect of liquid and paste-type lubricants on torque values during simulated rotary root canal instrumentation. *Int Endod J*. 2005 Apr;38(4):223-9.
 17. Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indications for their use. *Int Endod J*. 2003 Dec;36(12):810-30.
 18. Li X, Wu T, Bao H, Liu X, Xu C, Zhao Y, *et al*. Potential toxic trace element (pte) contamination in baoji urban soil (NW China): Spatial distribution, mobility behavior, and health risk. *Environ Sci Pollut Res*. 2017;24(24):19749-66.
 19. Lottanti S, Gautschi H, Sener B, Zehnder M. Effects of ethylenediaminetetraacetic, etidronic and peracetic acid irrigation on human root dentine and the smear layer. *Int Endod J*. 2009;42(4):335-43.
 20. Chassot ALC, Poisl MIP, Samuel SMW. *In vivo* and *in vitro* evaluation of the efficacy of a peracetic acid-based disinfectant for decontamination of acrylic resins. *Braz Dent J*. 2006;17:117-21.
 21. Barcellos DPDC, Farina AP, Barcellos R, Souza MA, Borba M, Bedran-Russo AK, *et al*. Effect of a new irrigant solution containing glycolic acid on smear layer removal and chemical/mechanical properties of dentin. *Sci Rep*. 2020;10(1):7313.
 22. Fischer T, Perosino E, Poli F, Viera M, Dreno B. Cosmetic dermatology european expert group. Chemical peels in aesthetic dermatology: An update 2009. *J Eur Acad Dermatol Venereol*. 2010;24(3):281-92.
 23. Fartasch M, Teal J, Menon G. Mode of action of glycolic acid on human stratum corneum: Ultra structural and functional evaluation of the epidermal barrier. *Arch Dermatol*. 1997;289(7):404-9.
 24. Cecchin D, Farina AP, Vidal CM, Bedran-Russo AK. A novel enamel and dentin etching protocol using α -hydroxy glycolic acid: Surface property, etching pattern, and bond strength studies. *Oper Dent*. 2018;43(1):101-10.
 25. Cecchin D, Brighenti IL, Bernardi JB, Leal LO, Souza MA, Bedran-Russo AK, *et al*. Alpha-hydroxy glycolic acid for root dentin etching: Morphological analysis and push out bond strength. *Int J Adhes Adhes*. 2019;90:138-43.
 26. Hua X, Cao R, Zhou X, Xu Y. One-step continuous/semi-continuous whole-cell catalysis production of glycolic acid by a combining bioprocess with in-situ cell recycling and electro dialysis. *Bioresour Technol*. 2019;273:515-20.
 27. Murphy SK, Zeng M, Herzon SB. A modular and enantio selective synthesis of the pleuromutilin antibiotics. *Science*. 2017;356(6341):956-9.
 28. Kakudo N, Kushida S, Suzuki K, Kusumoto K. Effects of glycolic acid chemical peeling on facial pigment deposition: Evaluation using novel computer analysis of digital-camera-captured images. *J Cosmet Dermatol*. 2013;12(4):281-6.
 29. Vemuri S, Kolanu SK, Varri S, Pabbati RK, Penumaka R, Bolla N. Effect of different final irrigating solutions on smear layer removal in apical third of root canal: A scanning electron microscope study. *J Conserv Dent*. 2016;19(1):87-90.
 30. Gambin DJ, Leal LO, Farina AP, Souza MA, Cecchin D. Antimicrobial activity of glycolic acid as a final irrigant solution for root canal preparation. *Gen Dent*. 2020;68(1):41-4.
 31. Zmener O, Pameijer CH, Banegas G. Effectiveness in cleaning oval-shaped root canals using anatomic endodontic technology, profile and manual instrumentation: A scanning electron microscopic study. *Int Endod J*. 2005;38(6):356-63.
 32. Peters OA, Schönenberger K, Laib A. Effects of four Ni–Ti preparation techniques on root canal geometry assessed by micro computed tomography. *Eur. J. Dent*. 2001 Apr;34(3):221-30.
 33. Mohammadi Z, Jafarzadeh H. The oval shaped root canal: a clinical review: Clinical review. *S. Afr. Dent. J*. 2015 Jun 1;70(5):200-4.
 34. Cleghorn BM, Christie WH. Morphology of Teeth and Their Root Canal Systems. *Ingle's Endodontics 7th ed.*, PMPH, USA; c2019. p. 53-4.
 35. Capar ID, Arslan H, Akcay M, Uysal B. Effects of ProTaper Universal, ProTaper Next, and HyFlex instruments on crack formation in dentin. *J. Endod*. 2014 Sep 1;40(9):1482-4.
 36. Souza MA, Palhano HS, Macci D, Broch GM, Tissiani L,

- Rauber BF, *et al.* Influence of ultrasonic activation on antimicrobial activity of a new final irrigant containing glycolic acid: An *in vitro* study. *Aust Endod J.* 2021;47(3):531-7.
37. Ghobashy A, Nagy MM, Obeid MF. Shaping ability of single versus multi file rotary Ni-Ti systems in curved root canals. *Tanta Dent. J.* 2016 Apr 1;13(2):68-72.
 38. Hecker H, Bartha T, Löst C, Weiger R. Determining the apical preparation size in premolars: part III. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2010 Jul 1;110(1):118-24.
 39. Usman N, Baumgartner JC, Marshall JG. Influence of instrument size on root canal debridement. *J. Endod.* 2004 Feb 1;30(2):110-2.
 40. Boutsoukis C, Kastrinakis E, Lambrianidis T, Verhaagen B, Versluis M, van der Sluis LW. Formation and removal of apical vapor lock during syringe irrigation: a combined experimental and Computational Fluid Dynamics approach. *Int. Endod. J.* 2014 Feb;47(2):191-201.
 41. Singla M, Aggarwal V, Logani A, Shah N. Comparative evaluation of rotary ProTaper, Profile, and conventional stepback technique on reduction in *Enterococcus faecalis* colony-forming units and vertical root fracture resistance of root canals. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2010 Mar 1;109(3):e105-10.
 42. Kumar M, Sequeira PS, Peter S, Bhat GK. Sterilisation of extracted human teeth for educational use. *Indian J. Med. Microbiol.* 2005 Oct 1;23(4):256-8.
 43. Roshdy NN, Kataia EM, Helmy NA. Assessment of antibacterial activity of 2.5% naocl, chitosan nanoparticles against enterococcus faecalis contaminating root canals with and without diode laser irradiation: An *in vitro* study. *Acta Odontol Scand.* 2019;77(1):39-43.
 44. Paisano A, Spira B, Cai S, Bombana AC. *In vitro* antimicrobial effect of bacteriophages on human dentin infected with enterococcus faecalis atcc 29212. *Oral Microbiol Immunol.* 2004;19(5):327-30.
 45. Elkabbany A. Cumulative Impacts of Endodontic Procedures on *Enterococcus Faecalis*: An *In vitro* Study. *Egypt. Dent. J.* 2022 Apr 1;68(2):1967-78.
 46. Saxena D, Saha SG, Saha MK, Dubey S, Khatri M. An *in vitro* evaluation of antimicrobial activity of five herbal extracts and comparison of their activity with 2.5% sodium hypochlorite against *Enterococcus faecalis*. *Indian J Dent Res.* 2015 Sep 1;26(5):524-7.
 47. Vaziri S, Kangarlou A, Shahbazi R, Nasab AN, Naseri M. Comparison of the bactericidal efficacy of photodynamic therapy, 2.5% sodium hypochlorite, and 2% chlorhexidine against enterococcus faecalis in root canals; an *in vitro* study. *J Dent Res Dent.* 2012;9(5):613-8.
 48. Dioguardi M, Di Gioia G, Illuzzi G, Laneve E, Cocco A, Troiano G. Endodontic irrigants: Different methods to improve efficacy and related problems. *Eur J Dent* 2018;12(03):459-66.
 49. Zeid STA, Bastawy HA, Saleh AAM. Natural extracts as biological smear layer removing agents: A literature review. *J Int Soc Prev Community Dent.* 2021;11(6):589-600.
 50. Mendonça DH, Colucci V, Rached-Junior FJ, Miranda CE, Silva-Sousa YT, SILVA SR. Effects of various irrigation/aspiration protocols on cleaning of flattened root canals. *Braz. Oral Res.* 2015 Jul 3;29:1-9.
 51. Kirk S, Skepper J, Donald A. Application of environmental scanning electron microscopy to determine biological surface structure. *J Microsc.* 2009;233(2):205-24.
 52. Collins SP, Pope RK, Scheetz RW, Ray RI, Wagner PA, Little BJ. Advantages of environmental scanning electron microscopy in studies of microorganisms. *Microsc Res Tech* 1993;25(5-6):398-405.
 53. Fitzek H, Schröttner H, Wagner J, Hofer F, Rattenberger J. Experimental evaluation of environmental scanning electron microscopes at high chamber pressure. *J Microsc.* 2015;260(2):133-9.
 54. Zmener O, Pameijer CH, Serrano SA, Palo RM, Iglesias EF. Efficacy of the navitip fx irrigation needle in removing post instrumentation canal smear layer and debris in curved root canals. *J Endod.* 2009;35(9):1270-3.
 55. Rüttermann S, Virtej A, Janda R, Raab WH-M. Preparation of the coronal and middle third of oval root canals with a rotary or an oscillating system. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2007;104(6):852-6.
 56. Ahlquist M, Henningsson O, Hultenby K, Ohlin J. The effectiveness of manual and rotary techniques in the cleaning of root canals: A scanning electron microscopy study. *Int Endod J.* 2001;34(7):533-7.
 57. Khademi A, Saatchi M, Shokouhi MM, Baghaei B. Scanning electron microscopic evaluation of residual smear layer following preparation of curved root canals using hand instrumentation or two engine-driven systems. *Iran Endod J.* 2015;10(4):236-9.
 58. Abdelgawad RA, Eid BM. Evaluation of debris extrusion and smear layer production following preparation using three different nickel titanium files. *Egypt Dent J.* 2018;64(10):3567-74.
 59. Pinheiro S, Araujo G, Bincelli I, Cunha R, Bueno C. Evaluation of cleaning capacity and instrumentation time of manual, hybrid and rotary instrumentation techniques in primary molars. *Int Endod J.* 2012;45(4):379-85.
 60. Abdel Hafez E, Soliman AH. Antibacterial effect of salvadora persica roots nanoparticles versus sodium hypochlorite as root canal irrigation. *Egypt Dent J.* 2019;65(1):831-6.
 61. Souza MA, Dias CT, Zandoná J, Hoffmann IP, Menchik VHS, Palhano HS, *et al.* Antimicrobial activity of hypochlorite solutions and reciprocating instrumentation associated with photodynamic therapy on root canals infected with enterococcus faecalis—an *in vitro* study. *Photodiagnosis Photodyn Ther.* 2018;23:347-52.
 62. Tennert C, Schurig T, Al-Ahmad A, Strobel SL, Kielbassa AM, Wrbas K-T. Antimicrobial influence of different root canal filling techniques in experimentally infected human root canals. *Quintessence Int.* 2017;48(4):273-80.
 63. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. *Braz Dent J.* 2014;216(6):299-303.
 64. Zhang R, Chen M, Lu Y, Guo X, Qiao F, Wu L. Antibacterial and residual antimicrobial activities against enterococcus faecalis biofilm: A comparison between edta, chlorhexidine, cetrimide, mtad and qmix. *Sci Rep.* 2015;5(1):12944
 65. Cecchin D, Farina AP, Souza MA, Albarello LL, Schneider AP, Vidal CMP, *et al.* Evaluation of antimicrobial effectiveness and dentine mechanical properties after use of chemical and natural auxiliary irrigants. *J Dent.* 2015;43(6):695-702.
 66. Estrela C, Estrela CR, Barbin EL, Spanó JCE, Marchesan MA, Pécora JD. Mechanism of action of sodium

- hypochlorite. Braz Dent J. 2002;13:113-7.
67. Zeng M, Murphy SK, Herzon SB. Development of a modular synthetic route to (+)-pleuromutilin, (+)-12-epi-mutilins, and related structures. J Am Chem Soc. 2017;139(45):16377-88.
68. Koivistoinen OM, Kuivanen J, Barth D, Turkia H, Pitkänen J-P, Penttilä M, *et al.* Glycolic acid production in the engineered yeasts *saccharomyces cerevisiae* and *kluveromyces lactis*. Microb Cell Factories. 2013;12(1):1-16.
69. Dal Bello Y, Porsch HF, Farina AP, Souza MA, Silva EJNL, Bedran-Russo AK, *et al.* Glycolic acid as the final irrigant in endodontics: Mechanical and cytotoxic effects. Mater Sci Eng C. 2019;100:323-9.
70. Hülsmann M, Rummelin C, Schäfers F. Root canal cleanliness after preparation with different endodontic handpieces and hand instruments: a comparative SEM investigation. J Endod. 1997 May 1;23(5):301-6.
71. Paqué F, Luder H, Sener B, Zehnder M. Tubular sclerosis rather than the smear layer impedes dye penetration into the dentine of endodontically instrumented root canals. Int Endod J. 2006;39(1):18-25.
72. Ballal NV, Jain I, Tay FR. Evaluation of the smear layer removal and decalcification effect of qmix, maleic acid and edta on root canal dentine. J Dent. 2016;51:62-8.
73. Kato AS, Cunha RS, da Silveira Bueno CE, Pelegrine RA, Fontana CE, de Martin AS. Investigation of the efficacy of passive ultrasonic irrigation versus irrigation with reciprocating activation: An environmental scanning electron microscopic study. J Endod. 2016;42(4):659-63.

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